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The effect of microclimate on pig weight gain evaluated with multisensor

D. Farkas[1]
e-mail: farkas.domonkos@encosoft.hu

A. Kárpinszky[1]
e-mail: karpinszky.andras@encosoft.hu

I. Pógár[1]
e-mail: pogar.istvan@encosoft.hu

[1] EN-CO Software Zrt. Hungary, H-1113 Budapest, 77–79 Bocskai út

Abstract. In this study, a multisensory system was built to evaluate the effect of the temperature, humidity, the concentration of carbon dioxide and ammonia on pig weight gain. During the experiment, RGB-based image analysis provided body weight information of 22 pigs over a three-month period. In the experiment, two cameras were set to obtain pictures, and the resulting data showed high correlation. Pearson's and Spearman's correlation was calculated between the body weight and the monitored environmental parameters. Results showed that temperature negatively correlates with the body weight, while CO_2 and NH_3 have a positive correlation. In this study, humidity, random effect, and changes in temperature had slightly negative but not significant correlation with body weight gain. Multiple linear regression showed that temperature and humidity had a significant effect on the body weight gain of the pigs, while the effect of the NH_3 was also noticeable. Our results proved that image-analysis-based weight evaluation is a powerful tool in precision livestock farming and that environmental conditions have a significant effect on pigs' production.

Keywords and phrases: environmental data, remote sensing, RGB image analysis

1. Introduction

The daily weight gain of pigs is a multifaceted phenomenon influenced by a complex interplay of various factors. Among these determinants, genetics and feeding technology have seen remarkable advancements and innovations over the past few decades, playing pivotal roles in shaping the overall weight gain trends among pigs. However, there exists a multitude of less conspicuous variables that may exert substantial influence on pigs' weight gain trajectory (*Patience et al.*, 2015; *Wu et al.*, 2017).

Two such pivotal factors that have traditionally received less attention are the quality of the air within the pigpen and the temperature maintained inside the facility. While genetics and feeding technology are undoubtedly influential, the significance of environmental conditions in the pig farming industry cannot be either overstated or underestimated. The air quality within the pen, including variables such as carbon dioxide and ammonia levels, also humidity, is intrinsically linked to the health and well-being of the pigs. Likewise, the ambient temperature plays a pivotal role in regulating their metabolism and overall comfort (*Costa et al.*, 2013; *Hoha et al.*, 2013). These environmental parameters have often been overlooked in the broader context of weight gain, primarily due to a scarcity of comprehensive data.

Recognizing the dearth of information in this critical aspect, our research endeavours led us to develop a sophisticated multisensor system. This innovative technology was meticulously engineered to capture a wealth of environmental data, thus providing us with invaluable insights into the hitherto unexplored relationship between these environmental factors and the weight gain of pigs. In addition to the fundamental measurements of carbon dioxide, ammonia, and humidity levels in the air, our multisensor also recorded temperature variations. To ensure the credibility of our findings, we included wind strength as a control variable, given its obviously negligible influence on weight gain.

An appropriate ventilation and heating system within the pigpen is pivotal in maintaining these environmental variables within an optimal range. The optimization of air quality and temperature is a cornerstone of efficient pig farming practices, and our multisensor empowers us to monitor, analyse, and ultimately enhance these crucial parameters. Through the amalgamation of cutting-edge technology and the invaluable experiences of pig farming, this study seeks to shed light on the intricate and often overlooked connections between environmental conditions and the weight gain of pigs. The knowledge garnered from this research promises to revolutionize the pig farming industry, contributing to healthier and more sustainable practices for farmers and pigs alike.

2. Materials and methods

Experimental design

The experimental setup was conducted at a private farm situated in Németkér, Tolna County in the Transdanubian region of Hungary. The study involved pigs of DanBred genetics and focused on a single pig-fattening cycle. This cycle

encompassed the care and monitoring of 22 pigs confined within a single pen, commencing when the pigs were approximately three months old, with an initial weight of approximately 30 kilograms. The study spanned a three-month duration, concluding when the pigs' weights reached an average of cc. 115 kilograms. To measure the pigs' daily weight gain, we employed a non-invasive method using RGB-based image analysis according to two cameras (cam1 and cam2) (*Kárpinszky & Dobsinszki*, 2023).

Applied sensors

After conducting an exhaustive survey of the available products in the market, we meticulously handpicked a set of sensors best suited for gauging various environmental parameters in our study. The cornerstone of our selection criteria revolved around the need for sensors with a sufficiently broad and well-quantified measurement range, coupled with a moderate degree of accuracy and affordability. We firmly believed that precision was vital; nevertheless, we recognized the importance of practicality in sensor capabilities. For instance, while the ability to measure temperature with a resolution of a hundredth of a Kelvin is undoubtedly impressive, it proved excessive for our specific research objectives, whereas room temperature operation was essential.

Our temperature and humidity sensors, which serve as integral components of our multisensor system, led us to the SHT-30 device. What makes it an excellent and convenient choice is its dual functionality, allowing us to simultaneously measure both relative humidity with a 2 per cent precision and temperature with an accuracy of 0.5 Kelvin. The device also comes equipped with a protective cover, not only ensuring the sensor's safety but also streamlining the integration process with other components. To facilitate seamless communication with the outside world, the SHT-30 uses the I2C interface, and an Adafruit Library is readily available to enhance its compatibility.

Ammonia levels were measured with the MQ-137 sensor, an outstanding choice given its expansive measurement range spanning from 5 to 500 ppm. Operating on a 5V supply, this sensor provides an analogue output linearly correlated with the concentration of ammonia that it detects. Although it necessitates a 48-hour warm-up period, this feature is typical among ammonia sensors.

Carbon dioxide levels, on the other hand, were effectively monitored using the MH-Z16 sensor. With the capability to precisely gauge concentrations from 0 to 5,000 ppm, this sensor's 1 ppm quantization and a 5 per cent accuracy further enhanced its suitability for our purposes. Moreover, its short warm-up time of just three minutes and a 5V operation with a maximum current consumption of 150 mA added to its practicality. Communication with the external systems was facilitated through a serial UART line.

For measuring wind speed, we employed the ADA-1733 anemometer, which boasts a measurement range spanning from 0.5 m/s to 50 m/s. The sensor provides data with a 0.1 m/s quantization and a worst-case error of 1 m/s. Similar to the ammonia sensor, it also offers an analogue output that is proportionate to the detected wind speed value. These sensors, collectively chosen after careful consideration, are integral to our multisensor setup (*Figure 1*), ensuring that we capture comprehensive and precise data to shed light on the intricate relationship between environmental factors and pig weight gain.

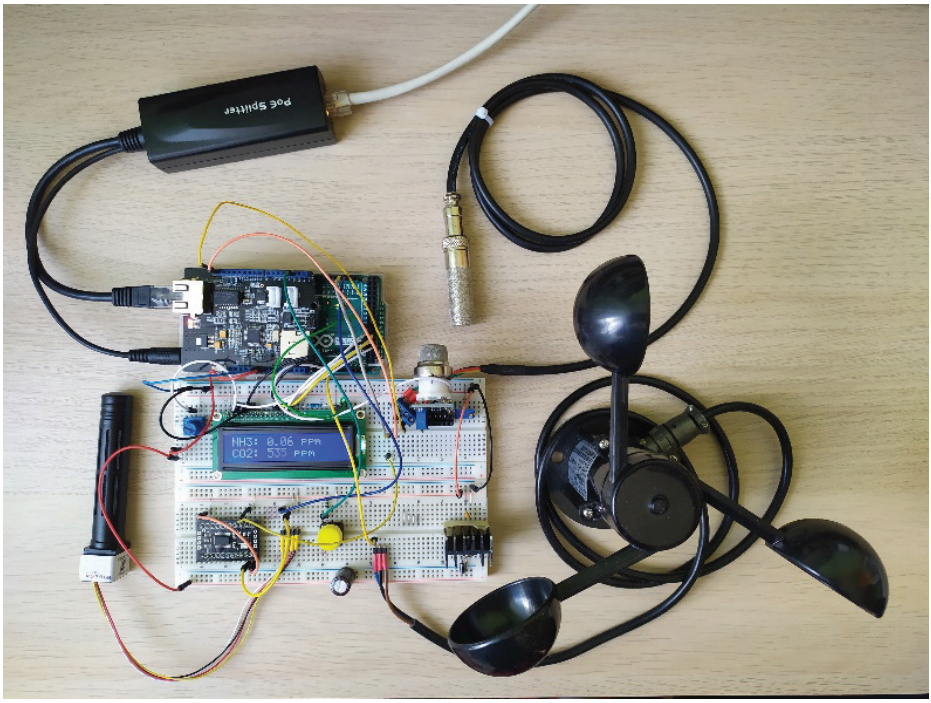

Figure 1. Multisensor test panel applied in this study

Data capturing and management

Regarding the physical infrastructure of our data management system, we selected the Arduino Mega as the central processing unit responsible for direct communication with the sensors (*Figure 2*). The Arduino was configured to transmit all measurement data via Power over Ethernet (PoE). To facilitate this communication, an A2971 Ethernet Shield was seamlessly integrated into the Arduino setup. The PoE connection was then extended to a Raspberry Pi, which served as the intermediary responsible for uploading the collected measurements to our designated web server. To ensure efficient communication and power supply

separation, a PoE switch was incorporated. Furthermore, a two-line LCD screen was linked to the Arduino to facilitate easy operational monitoring, allowing us to verify the system's functionality. This screen – although limited in its display capacity – offered the flexibility to toggle between different measurement types, using a dedicated button.

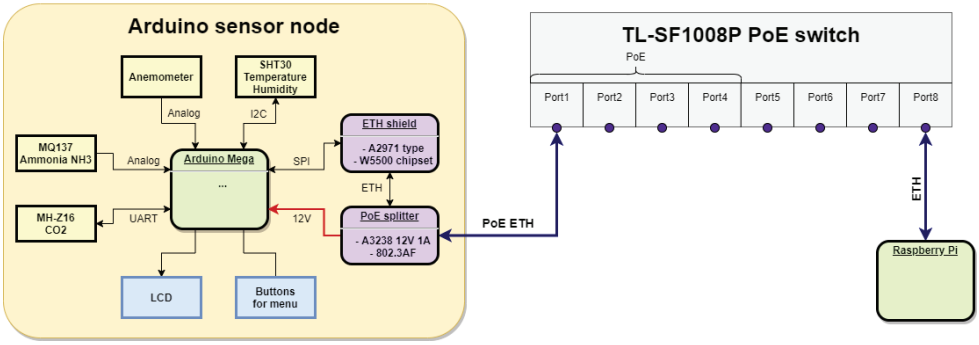

Figure 2. Physical setup of the system

In terms of software, we implemented sensor-specific readout interfaces within the Arduino framework, ensuring that data from various sensors could be harmoniously forwarded in a uniform and standardized format. Raspberry Pi played a pivotal role in the data transmission process, employing RabbitMQ and Masstransit to upload the data to our designated web server. The server, in turn, effectively stored the incoming data within a well-organized database structure and provided accessible endpoints for querying this information. Importantly, our data structures were designed to maintain flexibility, allowing for the seamless introduction of new measurement types. Each piece of data was tagged with its originating site identification, ensuring a comprehensive and organized record.

In terms of the quantitative aspects, our data collection system encompassed five sensors, with measurements taken at regular intervals (every five minutes). This extensive data collection initiative spanned from 22 November 2020 to 29 September 2021, resulting in the accumulation of a substantial 568,915 measurement values, equating to 113,783 measurements per sensor. Notably, the inclusion of pig weight data commenced on 29 June 2021, within which we recorded 132,415 measurements, thus 26,483 measurements per sensor. Given that pig weight data was computed on a daily basis, we consistently averaged the sensor measurements by day. As a result, we obtained a comprehensive 93-day dataset, in which pig weight and the corresponding averaged sensor values were meaningfully integrated and harmonized.

Assembling the sensors

The assembly of the sensors and computing devices underwent a meticulous and iterative process to ensure robustness and functionality. Initially, we established a proof-of-concept setup, illustrated in *Figure 1* (multisensor test panel), which successfully integrated all vital components. However, it became apparent that this initial setup lacked the required portability, stability, and resilience necessary to withstand the demanding conditions of our experiment.

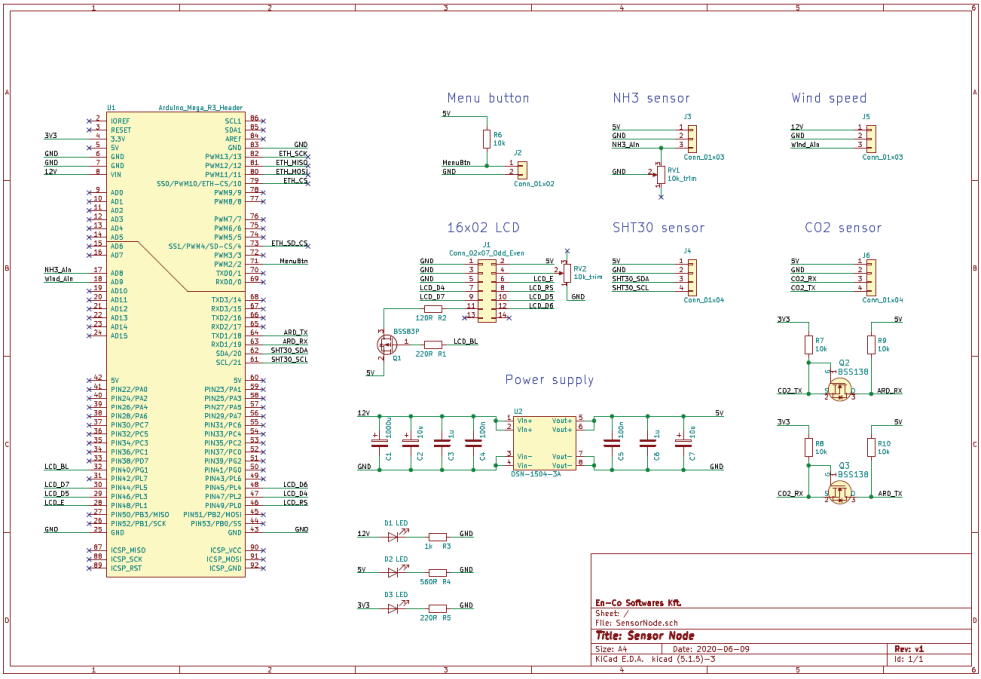

Figure 3. Wiring diagram

In our pursuit of a more durable configuration, we adopted a series of enhancements. First, we designed, fabricated, and seamlessly integrated a printed circuit board (*Figure 3*) using the KiCAD software and secured the electrical connections through soldering. Subsequently, we calculated the minimum dimensions required for an enclosure capable of accommodating all components, excluding the Raspberry Pi, which remained external to the enclosure. Our choice was the Hammond 1598BK box. To ensure optimal functionality and longevity, we employed FreeCAD to model the enclosed multisensor assembly. This process minimized mechanical and cable stress and ensured that heat-producing components did not affect each other adversely. With the design

finalized, we 3D printed custom sockets and cut openings in the enclosure, ultimately resulting in the configuration (*Figure 4*).

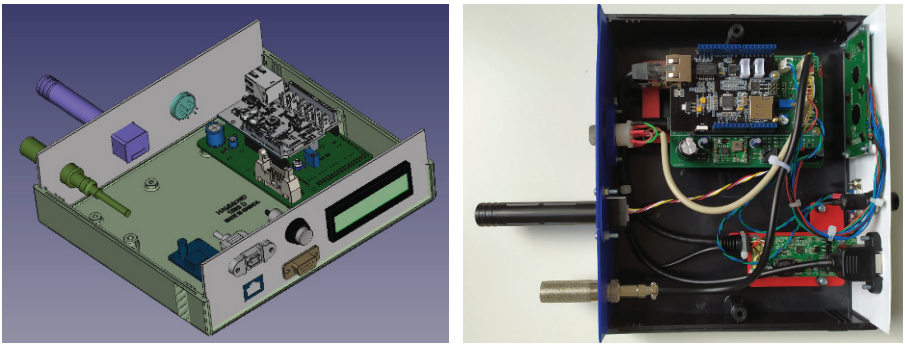

Figure 4. Boxed multisensor with FreeCAD model (left) and the physical realization (right)

At this juncture, we reached a stage where the multisensor setup was both portable and fully operational for testing. However, local testing revealed issues related to voltage stabilization, leading to overheating and occasional system shutdowns. To address this concern, we transitioned from our linear stabilizer to a switching voltage stabilizer, which significantly improved efficiency and so reduced heat generation due to power losses. This transition allowed the system to operate reliably for days without any sign of overheating.

Nonetheless, some heating persisted within the enclosure, decreasing the credibility of the temperature sensor. Consequently, we repositioned the temperature sensor outside the enclosure to ensure its proper functionality. Following these local tests and subsequent adjustments, the multisensor was deemed ready for an on-site trial.

Figure 5. Boxed multisensor on site: fresh (left), later (right)

The on-site evaluation highlighted the necessity for the enhanced protection of the sensors (*Figure 5*). Additionally, it became evident that the sensors needed to be positioned in closer proximity to the pigs to accurately measure the environmental parameters that directly influenced them. Achieving this proximity and the required protection posed new challenges.

To address these issues, we undertook the design and integration of a robust metal cover box of excellent air permeability. This new enclosure design not only facilitated valid measurements but also offered protection against the pigs, high-pressure washings, and other harsh environmental factors. Moreover, it ensured the safety of the pigs by preventing them from inadvertently causing harm to themselves. Consequently, this shielded multisensor configuration was placed inside the pigpen at the level of the pigs, as shown in *Figure 6*.

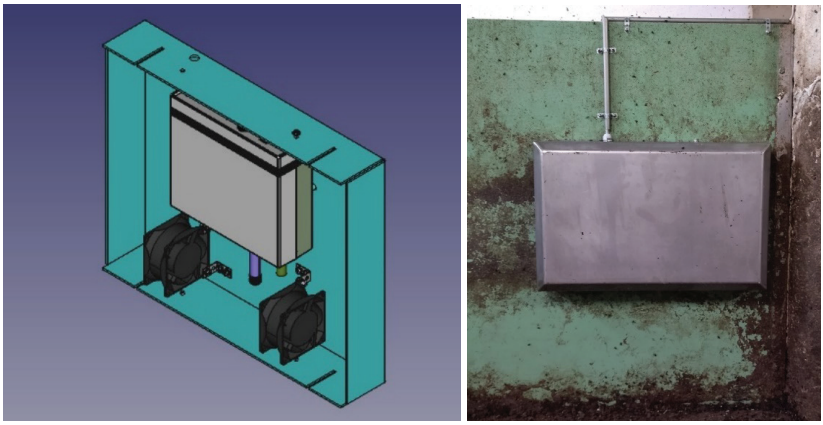

Figure 6. Shielded multisensor according to the FreeCAD model (left) and physical realization (right)

Statistical methods

We had two cameras above the pen; therefore, we obtained two weight series ("cam1" and "cam2") for the same pig population. We treated these two series independently. To improve the accuracy of average weight measurements, we applied a moving average filter on the raw average daily weight series. We computed the average daily weight gain by differentiating this smoothed weight series, so the average daily (smoothed) weight gain was as follows:

$$dw_{smooth}(k) = w_{smooth}(k) - w_{smooth}(k-1), \tag{1}$$

where: $w_{smooth}(k)$ is the smoothed average weight at day k.

Substituting the moving average formulas:

$$dw_{smooth}\left(k\right)=\frac{w\left(k-\frac{l}{2}\right)+\ldots+w\left(k+\frac{l}{2}\right)}{l}-\frac{w\left(k-\frac{l}{2}-1\right)+\ldots+w\left(k+\frac{l}{2}-1\right)}{l}, \quad (2)$$

in which internal addends cancel out, and so it simplifies to:

$$dw_{smooth}\left(k\right)=\frac{w\left(k+\frac{l}{2}\right)-w\left(k-\frac{l}{2}-1\right)}{l}. \quad (3)$$

Thus, the obtained average daily weight gain is equal to the difference of two days symmetrically further apart, divided by the number of days between them. For the latter calculations, we used a fifteen-day window.

Then pairwise correlation methods were applied to measure the relationship between each sensor data and the average daily weight gains of pigs. Both Pearson's and Spearman's correlation coefficients were calculated. The Pearson's method assesses the linear property of the relationship, while Spearman's correlation coefficient shows the extent of monotonic association between the variables. Both coefficients have ranges between -1 (perfectly negative correlation) and 1 (perfectly positive correlation). Multiple regression was also run to find an appropriate model for pig weight gain. To calculate them, we used Microsoft Excel's built-in functions, and the PAST software package (*Hammer & Harper*, 2001) was also applied.

3. Results and discussions

Pig production is one of the most important sectors of livestock farming in Hungary. With 2.7 million animals in 2021, Hungary ranked 11th among the EU27 countries (*KSH*, 2023a). According to the Hungarian Central Statistical Office, the per capita annual pork consumption in 2021 was 30.2 kg. This consumption was served by 3.2 million tons of national production and 1.7 million tons of import (*KSH*, 2023b). *Baráth et al.* (2021) showed that while from 2004 to 2019 pig production values and the number of specialized farms together with the number of animals decreased, whereas the average number of animals per holding increased, with many using precision farming technologies on the pig farms. *Kopler et al.* (2023) reviewed the most important pig-production-related precision-livestock-farming technologies and programs, including camera technology, microphones, animal-attached sensors, among them: environmental sensory thermometers, anemometers, and weather station. In our study, a multisensory

system was developed and applied to find correlation between the environmental circumstances and the daily weight gain of the investigated pigs.

Pig weight evaluation based on image analysis

Regular evaluation of pig weight gain is essential to obtain information about the physiological and health status of the animals. However, since the traditional procedure of weight measurement would be stressful for the animals, remote sensing methods have become increasingly widespread (*Kongsro*, 2014; *Li et al.*, 2014). Our former study showed that animals' biometric parameters obtained from RGB images are appropriate to predict animal weight (*Kárpinszky & Dobsinszki*, 2023). In this study, the weight gain of 22 pigs was monitored with RGB-based image analysis over a period of approximately 3 months. Two monitors were collecting data concurrently. The average initial weight of the pigs was 33.47 kg and 34.11 kg, resp., while the final weight on 29 September (the date up to which the sensor data were collected) was 115.29 kg and 115.53 kg, resp., according to the images obtained from the two cameras. Pearson's correlation coefficient of the weight and smoothed rolling average weight collected by the two cameras was 0.9999 ($p < 0.01$) for both. The lowest average daily weight gains were 0.5883 kg (from 27 to 28 September 2021) and 0.5187 kg (from 21 to 22 September 2021), while the highest were 1.63 kg and 1.96 kg (from 5 to 6 September 2021) according to cam1 and cam2 respectively (*Table 1*).

Microclimatic data evaluation

Hu et al. (2022) reviewed the importance of air quality in modern livestock husbandry, highlighting the thermal environment as a significant factor affecting pigs' health and production rate. At the same time, providing the optimal microclimate is a multiple task because of the high energy prices and the harmfulness of the environmental sensors. *Yeo et al.* (2023) detail the optimal location of the pig house sensors, where maintenance and minimizing sensor damage were also considered among the necessary conditions. In this research, microclimatic data were obtained with a multisensor system developed in this study to obtain long-term data in the pig house in 5-minute intervals. Daily average data of temperature, humidity, CO_2, and NH_3 were collected. The mean temperature during the experiment was 24.88 °C (min.: 18.42 °C, max.: 31.08 °C), with an average of -0.08 °C temperature change between two days (min.: -7.14 °C, max.: 4.2 °C). Humidity was between 51.95% and 78.39%, with the mean value of 62.69%. CO_2 concentration ranged from 387.05 ppm to 900.77 ppm, with the mean value of 601.85 ppm. Ammonia concentration was between 0 and 0.012 ppm, with 0.002 ppm on average (*Table 1*).

Table 1. Summary statistics of pig weight, pig weight gain (n = 22) obtained from the two cameras and environmental data collected with the multisensory system

	Mean	**St. dev.**	**Min.**	**Max.**
Pig weight (cam1) (kg)	71.54	24.11	33.47	115.29
Pig weight (cam2) (kg)	72.62	23.72	34.11	115.53
Daily weight gain (cam1) (kg)	0.9321	0.2166	0.5883	1.6336
Daily weight gain (cam2) (kg)	0.9362	0.2303	0.5187	1.9664
Daily weight gain (cam. aver.) (kg)	0.9341	0.2147	0.5741	1.8
Temperature	24.88	2.82	18.42	31.08
Humidity	62.69	6.51	51.95	78.39
CO_2	601.85	118.47	387.05	900.77
NH_3	0.0021	0.0023	0	0.012
Random	$1.28*10^{-6}$	$4.18*10^{-6}$	0	$2.37*10^{-5}$
Change of temperature	-0.08	2.04	-7.14	4.2

Correlation of the microclimatic data with pig weight gain

Previous studies have shown that environmental conditions have significant consequence on pig weight gain. *Rauw et al.* (2020) investigated the effect of different temperature settings during the growing, fattening, and finishing stages on body weight gain, feed intake, and feed efficiency. Their results showed significant differences on the monitored growth curve parameters influenced by the environmental groups.

In our study, temperature, change of temperature, humidity, CO_2 and NH_3 concentration were monitored to find correlation with the daily weight gain of the animals (*Table 2*). In general, Spearman-type correlations were slightly elevated compared to Pearson's coefficients, implying that the connections were not linear.

Results showed that temperature had a significantly ($p < 0.01$) ("p" is the *probability value* of the statistical model) negative Pearson's and Spearman's correlation with daily body weight gain. This finding shows that pigs' food consumption is lower at higher temperatures. It must be highlighted that the experiment was conducted starting from midsummer. Results pointed to the same direction in the case of weight data obtained from both cam1 and cam2 images.

CO_2 concentration showed significantly ($p < 0.01$) positive correlation with body weight gain. This is probably because weightier pigs tend to exhale more CO_2 and gain more weight in absolute terms. On the other hand, in this interval, the amount of carbon dioxide was not enough to significantly prevent the pigs from gaining more weight. The NH_3 concentration also weakly correlated positively ($p < 0.01$) with body weight gain. Likewise, this result may be attributable to the metabolism

of weightier pigs. The ventilation system was good enough to maintain a healthy concentration of NH_3.

Neither humidity nor temperature change nor random effect has a significant correlation with the collected weight data. Unsurprisingly, the random effect showed very low negative correlations (not significant). It also greatly fluctuated if we changed the moving average window size. For this calculated feature, computations resulted in no or only very week correlation. Multiple linear regression calculated by the average body weight gain values resulted from the 2 cameras showed significance, and the model showed that temperature and humidity had a significant effect ($p < 0.01$) on the body weight gain of the pigs, while the p value of the NH_3 concentration was 0.05 (*Table 3*).

The MLR model is (n = 86, multiple R = 0.57, multiple $R^2 = 0.33$):

$$Y = 3.29 - 0.04x_1 - 0.01x_2 - 0.0004x_3 + 24.83x_4 - 3407.1x_5 - 0.0032x_6, \quad (4)$$

where x_1 refers to temperature, x_2 refers to humidity, x_3 refers to CO_2, x_4 refers to NH_3, x_5 is the random effect, and x_6 refers to the change of temperature.
The F-test value was 6.53 on df1, df2: 6, 79 ($p < 0.01$).

Table 2. Pearson's correlation of the environmental parameters with the average daily weight gain (moving average window size: 15)

Sensor data type	**Pearson's correlation coeff.**	
	cam1	cam2
Temperature	-0.4337*	-0.3985*
Humidity	-0.0615ns	-0.0863ns
CO_2	0.3338*	0.3130*
NH_3	0.3451*	0.3101*
Random	-0.0659ns	-0.1084ns

Note: * indicates significant correlation between the monitored environmental parameter and daily body weight gain at $p < 0.01$.

Table 3. Multiple linear regression model

Sensor data type	**Coeff.**	**Std. err.**	**t**	**p**	**R^2**
Temperature (x_1)	-0.04	0.01	-3.79	0.00	0.18
Humidity (x_2)	-0.01	0.00	-4.00	0.00	0.00
CO_2 (x_3)	0.00	0.00	-1.33	0.18	0.11
NH_3 (x_4)	24.83	12.75	1.94	0.05	0.11
Random (x_5)	-3407.1	5131.9	-0.66	0.50	0.00
Change of temperature (x_6)	0.00	0.01	-0.31	0.75	0.00

4. Conclusions

In this study, RGB-based remote sensing was applied to collect information about daily pig weight gain. A recently developed multisensor system was applied to collect environmental information about temperature, humidity, the concentration of carbon dioxide and ammonia. We found that temperature has a negative correlation with pig body weight gain, while the correlation was positive concerning the CO_2 and NH_3 concentration.

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Zooming into CTC black-tea wine metabolites: A GC-MS-based study

S. Majumder[1]
e-mail: soumyamajumder777@gmail.com

A. Ghosh[1]
e-mail: arindamghosh611@gmail.com

S. Chakraborty[1,2]
e-mail: srvjalac@gmail.com

M. Bhattacharya[1]
e-mail: malaytsnbu@gmail.com

[1] Molecular Biology and Tissue Culture Laboratory, Department of Tea Science, University of North Bengal, Siliguri, Darjeeling 734013, West Bengal, India
[2] Postgraduate Department of Botany, Darjeeling Government College, Darjeeling 734101, West Bengal, India

Abstract. This research was designed to propose a report on the fermentation metabolomics of CTC (crush-tear-curl) tea wine (TW), a yeast-fermented broth of sugared CTC black tea infusion. The gas chromatography-mass spectrometry analysis of the tea wine revealed the presence of thirty-five metabolites, including the major compound glycerine with some potential antioxidant molecules and other bioactive agents (4H-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-; furfural; furfuryl alcohol; succinic acid; levulinic acid; palmitic acid; tyrosol, pyruvaldehyde; and 1-hexadecanol). The role of metabolites in the physicochemical, biochemical, and medicinal properties of TW has been discussed. Biomolecules responsible for the flavour of TW were as follows: glycerine derivatives; pyruvaldehyde; furfural; furfuryl alcohol; acetic, levulinic, succinic, and palmitic acids, etc. – which might develop a sweet, caramel-like, astringent, slightly sour and wine-like flavour and taste. Furthermore, on the basis of yeast metabolism, possible biosynthesis pathways of metabolites were designed aiming for fermentation metabolomics. The outcome of this study cross-verified physicochemical, biochemical, and medicinal properties of TW suggesting its acceptability. As the fields of both wine research and tea science continue to evolve, the findings of this study may encourage fermentation technology for product development from tea that may also boost the growth of the tea industry.

Keywords and phrases: CTC tea, tea wine, fermentation, bioactive metabolites, biosynthesis pathway

1. Introduction

Wines contain several metabolites derived from the substrates and the fermenting yeast, which together determine its quality. Wine's bioactivities have been associated with reduced risk of cardiovascular and oxidative-stress-related diseases, as wines are rich sources of polyphenolic antioxidant substances such as flavonoids, flavonols, anthocyanins, leuco-anthocyanins, catechins, and resveratrol (*German & Walzem*, 2000; *Villano et al.*, 2006). Additionally, wines possess various cardioprotective and neuroprotective medicinal properties such as: promoting smooth blood circulation; providing antioxidants to prevent the degradation of cell walls in arteries and the brain; contributing to the breakdown of blood platelets and the balance of fibrinolysis, which is essential for coagulation; helping in the treatment of cancer and Alzheimer's disease (*Arranz et al.*, 2012). Fermentation not only depends on the type of substrate material, but also there are influences exerted by the starter's microflora. The fermentation of wine naturally occurs through the introduction of inoculated starters or yeasts, which consists of beneficial strains. The quality of commercial wine yeast is determined by its oenological properties and parameters such as storage, stability, osmotolerance, freeze-thaw resistance, and drying/rehydration resistance. *Saccharomyces cerevisiae* is mostly the principle agent of wine fermentation to convert hexose sugars to ethanol, carbon dioxide, and a variety of compounds, including sugar alcohols, esters, aldehydes and acids, which contribute to the sensory attributes of the beverage (*Viljoen*, 2006). The technological process involves a wide range of yeast species making different contributions to wine quality. *Saccharomyces cerevisiae* and *Saccharomyces bayanus* are "wine yeasts", the most desired agents of wine fermentation, which have the highest oenological potential and are commonly used in wine making (*Braschi et al.*, 2019). There is a universal agreement that *S. cerevisiae* predominates in fermenting broth after a few days of spontaneous fermentation (*Braschi et al.*, 2019; *Majumder et al.*, 2022a).

Generally, fermented alcoholic beverages from different raw materials are called "wine" added with the name of that material. Therefore, the one prepared from tea can be termed as tea wine. Tea is globally the second most popular and consumed low-cost beverage, second only to water, and is taken for rejuvenation, relaxation, and health benefits. The average global consumption of tea, which is around 100 ml/head/day, is much ahead of coffee, beer, wines, and carbonated soft drinks (*Trevisanato & Kim*, 2000). There are many types of tea produced in the tea-growing regions of the world such as black tea, green tea, white tea, oolong tea, Pu-erh tea, etc. Among them, black tea is a good fermentation medium because the infusion contains proteins, amino acids, volatile compounds, lipids, enzymes, and polyphenols in a good amount, which also gives it a unique flavour (*Kumar & Joshi*, 2016; *Majumder et al.*, 2022a). The development of the ancient ethnic beverage kombucha and its emerging popularity evidenced that brewed

tea, being the most popular drink with medicinal properties, can be used as a potential substrate in fermentation technology. Kombucha is a fizzy, sour, flavoury, and less alcoholic fermented tea infusion brewed by a starter called SCOBY (the symbiotic colony of bacteria and yeast) (*Jayabalan et al.*, 2007; *Majumder et al.*, 2020). After ages of research and development on kombucha, the term "tea wine" was coined by oenologists, which refers to the wine made from tea (*Li et al.*, 2020). The fermentation of sugared broth of tea using wine yeast (*Saccharomyces cerevisiae*) or koji (*Aspergillus oryzae*, traditional rice wine starter) to produce a low alcoholic "tea wine" has been reported (*Li et al.*, 2020; *Majumder et al.*, 2022a). However, there is very little information available on the production and characterization of tea wine (*Majumder et al.*, 2022a) in the scholarly world, unlike the case of kombucha, which is a constantly top-trending topic in food science and food technology. Among the various innovations and technologies, microbial fermentation is an option that can be utilized to increase biological activities and alter the flavour profile of a typical cup of tea. Recent reports on tea wine, tea-flower wine, and production of bioactive formulations like "tea haria" by using the indigenous starter "bakhar" also justified the importance of this issue (*Majumder et al.*, 2021; *Majumder et al.*, 2022b). *Aroyeun et al.* (2005) established that their tea wine preparation has an excellent sensory profile with good flavour characteristics. The tea wine could be expected to not only serve as a mild stimulatory drink but also a health supplement with low alcohol content.

Mainly two types of tea are consumed, i.e. green tea and black tea (orthodox hand-rolled whole-leaf tea and crush-tear-curl, or CTC tea), in the world, which are mostly produced and exported by leading tea-growing nations such as China and India (*Majumder et al.*, 2022a). CTC, or "crush-tear-curl", is a type of manufactured black tea that is the most commonly manufactured and the most consumed type of tea worldwide (*Pou et al.*, 2019; *Majumder et al.*, 2022c). In the Indian domestic market, CTC tea is by far the most popular choice in tea shops and domestic households (over 80% of tea production is of this type), which is less expensive and mostly sold loose in markets of India (*Solanki*, 2022).

Our research group has already developed different fermented tea infusions from both black tea (CTC tea and orthodox black tea) and green tea, using brewer's yeast (*Saccharomyces cerevisiae*) and the traditional kombucha starter SCOBY (*Majumder et al.*, 2022a) and carried out a comparative *in vitro* biochemical characterization. Moreover, research results suggested CTC black tea as the most potential substrate for showing the highest fermentation-led increased antioxidant property, unlike orthodox black tea and green tea that already contain notable levels of bioactives in their raw/crude states (*Majumder et al.*, 2022a). Interestingly, fermented CTC tea samples exhibited comparatively higher alterations in physicochemical properties due to fermentation than other tea samples (*Majumder et al.*, 2022a). Therefore, unlike expensive orthodox or hand-rolled whole-leaf black tea and green tea, cheaper or less costly CTC tea was validated as the ideal solution to be utilized

in fermentation technology where fermentation-driven quality enhancement was found to be the highest. After preliminary *in vitro* experiments, it was crucial to investigate the metabolite profile before further *in vivo* tests and consumption. Therefore, in this follow-up research work, GC-MS analysis was utilized to explore the volatile profile of CTC-tea wine (TW). The objectives of this study were to conduct the metabolite profiling of TW through GC-MS analysis, correlate the results with known biochemical characteristics and bioactivities of TW, and elucidate the biosynthesis pathways of the identified metabolites.

2. Materials and methods

Collection of CTC-tea wine (TW) and sample preparation for GC-MS analysis

Fermented TW sample (*Figure 1*) was collected from the fresh batch (fifteen days of fermentation period), which was used for physicochemical and biochemical tests and *in vitro* bioactivity analysis (as reported in: *Majumder et al.*, 2022a). The brewing process has been reported as follows: ten grams of CTC tea (or 1% w/v) were added in one litre of freshly boiled double-distilled sterile water (at 98 ± 1 °C) and left for fifteen minutes to prepare the tea infusion (generally 2–5 minutes are preferred; here, brewing time was extended for the proper extraction of biomolecules, including caffeine into the broth or substrate). The infusion was then filtered through sterile muslin cloth and poured into a fermentation jar followed by adding 100 g of sucrose (or 10% w/v) as nutrient or carbon source for fermenting microbes. The jar was then autoclaved properly for sterilization. After cooling at room temperature, 2 g of dried brewer's yeast – *Saccharomyces cerevisiae* – was added as starter in the jar. Sterile acetic acid, or synthetic white vinegar, was added (to maintain the pH at 5) in the jar to increase the acidity of the fermentation broth (considered as an ideal fermentation condition). The mouth of the jar was covered with polythene sheet having pores to facilitate the gas (CO_2) release. Muslin cloth, glass goods, polythene cover, etc. were autoclaved and/or sterilized with 70% ethanol before using. The jar was then incubated in dark conditions at a well-ventilated and airy room for fifteen days. A control wine batch (CW) was prepared by inoculating 2 g of dried brewer's yeast and other ingredients in broths where tea infusion was replaced by sterile double-distilled water.

This is a follow-up metabolomic research on CTC-tea wine. In this research, the best performing (having the highest bioactive potential) and most healthy (the most uncontaminated) replicas of TW and CW samples were chosen for GC-MS analysis. One millilitre of both TW (*Figure 1: B*) and CW (*Figure 1: C*) were taken

in separate test tubes, air dried completely, and dissolved in 1 ml of methanol (chromatography grade from SRL, India) to prepare methanolic extracts prior to the GC-MS analysis.

Figure 1. A – fresh batch set-up for fermentation (CTC/Y = CTC-tea wine batch and C/Y = control wine batch), B – fermented CTC-tea wine (TW) sample, C – fermented control wine sample

GC-MS analysis

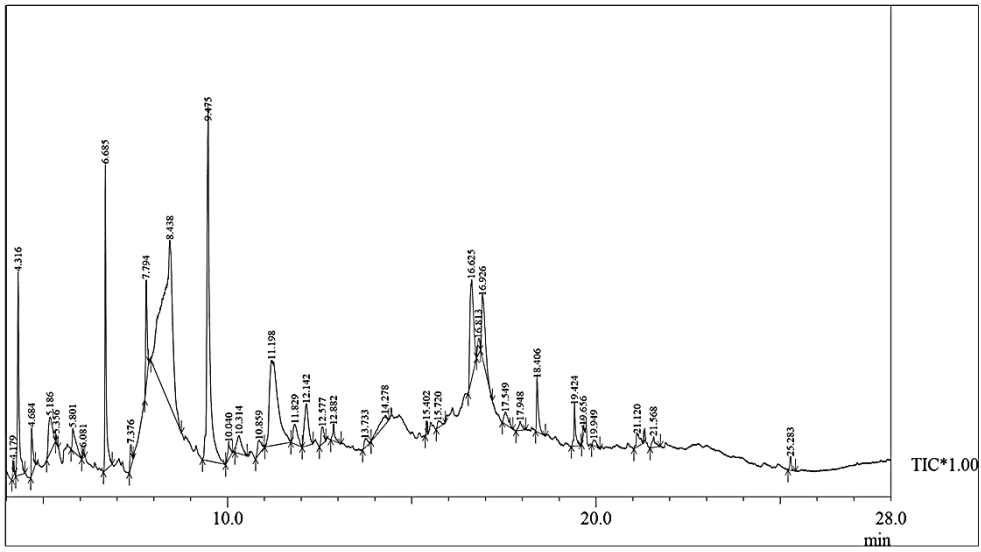
Samples (TW and CW) were subjected to GC-MS analysis following the pre-standardized research protocol for tea petal wine developed by *Majumder et al.* (2021). GCMS-QP2010 Plus (Shimadzu Co., Japan) with a DB-5 fused-silica capillary column (30 m × 0.25 mm × 0.25 µm) was used in this research. The split injection (with a ratio of 20:1) technique was adopted, where the injection volume was 1 µL. Column oven temperature: 80 °C, carrier gas: helium (99.9995% purity), injection temperature: 260 °C, total flow rate: 16.3 mL/min, and column flow rate: 1.21 mL/min were considered. Flow control mode was at a linear velocity of 40.5 cm/sec. The interface temperature and ion source temperature were set to 270 °C and 230 °C respectively. Total ion chromatogram (TIC) was based on the intensity of fragments produced by the ionization. A single-quadrupole mass spectrometer was used in this research. Mass spectra were recorded at 5 scan/sec with a scanning rate of 40-600 m/z. For compound identification, the probability-based matching method was used, and the obtained spectra were compared to the Wiley and the NIST databases. Data acquisition and control of the chromatograph were carried out using the GCMS solution software. According to the guidelines by Shimadzu Co., the peak area corresponds to the amount of compound present in a sample (https://www.shimadzu.eu.com), and previously researchers (*Acharyya et al.*, 2021; *Chakraborty et al.*, 2023) also considered the peak area for the quantification of detected compounds (relative area %). Based

on the results of the GC-MS analysis (peak report), the lists of metabolites were prepared. A further study was based on the available literature and databases. The results have been expressed in the following order: a. metabolite profile (with peak reports and chemical classification); b. role of metabolites in physicochemical and biochemical properties; c. medicinal properties of the metabolites; d. flavour-imparting molecules; e. metabolic pathways to study the metabolites' biosynthesis.

3. Results and discussions

Metabolite profile

The GC chromatograms of the sample TW and CW are presented in *Figure 2,* and the list of metabolites based on peak reports are included in *Table 1*. A total of thirty-five compounds have been detected in the TW sample, including control metabolite glycerol as a major component (glycerine- 26.6%; and glycerol, 1-acetate- 11.61%). Sugar alcohol glycerine derivatives occupied almost the whole chromatogram (95.72%) of the control or sample CW, which include triol-glycerine (86.43%), secondary alcohol- 2,3-butanediol (7.52%), and glycerol derivatives (cis-5-hydroxy-2-methyl-1,3-dioxane and 4-hydroxymethyl-2-methyl-1,3-dioxolane). The domination of compound glycerine was clearly reflected in the CW's chromatogram (*Figures 2B* and *3*), while the majority of the peaks of different molecules is visible in the sample TW (*Figures 2A* and *B*).



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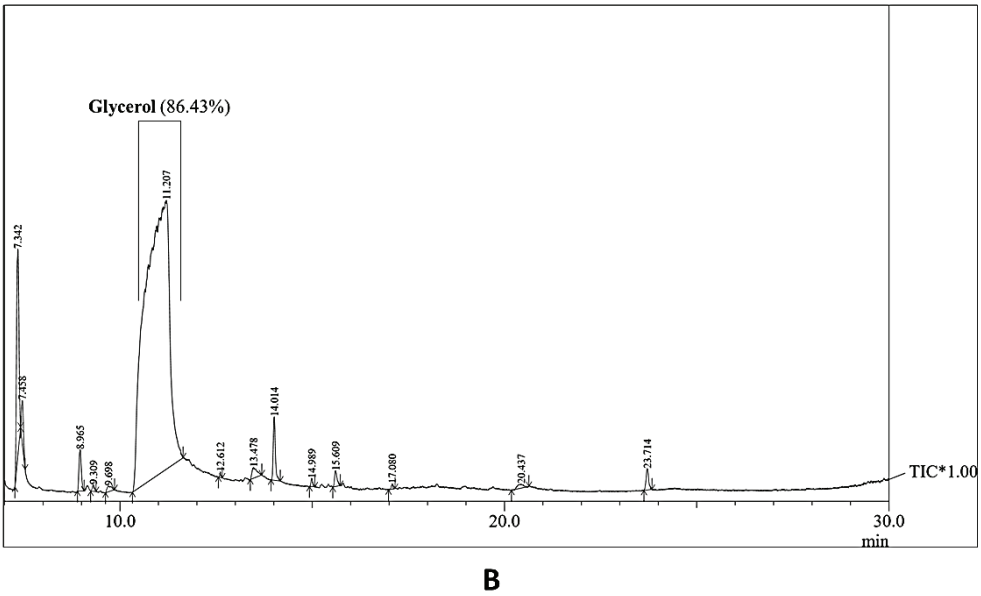


Figure 2. A – GC chromatogram of sample TW, B – GC chromatogram of sample CW (showing a large peak of glycerine)

Table 1. GC-MS peak reports derived list of metabolites for the sample CTC-tea wine (TW) and control wine (CW) and their peak area (%)

Name of the compound	Type of compound	TW	CW
3-Methyl-2(5H)-furanone	Furanone derivative	0.24	
Furfural	Furans	4.99	
Furfuryl alcohol	Furans	1.26	
Butanoic acid, 2-ethyl-, methyl ester	Carboxylic acid	2.31	
Pyruvaldehyde dimethyl acetal	Sugar aldehyde	0.12	
1,3-Cyclopentanedione	Cycloalkane	1.33	
(+-)-4-Amino-4,5-dihydro-2(3H)-furanone	Furanone derivative	0.18	
2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one	Furanone derivative	6.23	
Succinic acid derivative	Carboxylic acid	0.65	0.11
Levulinic acid	Carboxylic acid	2.49	
Glycerine	Sugar alcohol (triol)	26.6	86.43
4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	Pyrone (flavonoid fraction)	15.58	

Name of the compound	Type of compound	TW	CW
Cyclobutanecarboxylic acid, octadecyl ester	Carboxylic acid	0.61	
Isosorbide Dinitrate	Sugar alcohol (sorbitol) derivative	2.11	
Pyruvic acid ethyl ester	Carboxylic (keto) acid (etserified)	0.82	
Glycerol, 1-acetate	Acetic acid ester of sugar alcohol (triol)	11.61	
Acetic acid, hexyl ester	Carboxylic acid, fatty acid ester	1.41	
Octyl octanoate	Fatty acid derivative	2.68	
Tridecyl acrylate	Fatty acid derivative	0.53	
Tyrosol	Phenol	0.46	
1-Oxa-4-azaspiro[4.5]dec-4-yl-6,6,10,10-D4-oxy, 3,3-dimethyl		1.25	
1-Hexadecanol	Fatty alcohol	0.2	
Myrtanol	Monoterpenoid	0.35	
Pyrimidine-2,4(1H,3H)-dione, 6-hydroxy-5-methyliminomethyl-	Pyrimidine	5.7	
Octanoic acid, 4-pentadecyl ester	Fatty acid	0.48	
1-Isobutyl-7,7-dimethyl-1,3,4,5,6,7a-hexahydroisobenzofuran-3a-ol	Furans	3.57	
3-Butyl-4-nitro-pent-4-enoic acid, methyl ester	Carboxylic acid	0.86	
Oxalic acid, allyl octadecyl ester	Carboxylic acid (fatty acid) derivative	0.43	
Caffeine	Purine alkaloid	1.63	
Palmitic acid	Fatty acid	1.16	
Furfuryl heptanoate	Furan (fatty acid ester)	0.24	
trans-Ascaridol glycol	Monoterpenoid	0.32	
(3Z,9Z)-cis-6,7-epoxy-3,9-nonadecadiene	Fatty acid derivative	0.75	
Palmitic acid, ethyl ester	Fatty acid	0.51	
Phthalic acid derivative	Phthalate	0.33	0.71
2,3-Butanediol	Secondary sugar alcohol		7.52
cis-5-Hydroxy-2-methyl-1,3-dioxane	Glycerin derivative		1.27

Name of the compound	Type of compound	TW	CW
4-Hydroxymethyl-2-methyl-1,3-dioxolane	Glycerin derivative		0.5
Eucalyptol	Monoterpenoid		0.08
5-Acetyldihydrofuran-2(3H)-one	Furanone derivatives		0.66
Phenylethyl Alcohol	Primary alcohol		1.73
L-Camphor	Monoterpenoid		0.18
4-(1-hydroxyethyl)-gamma-butanolactone	Furanone derivatives		0.51
Phenylethylene glycol	Glycol		0.3

Role of metabolites in CTC-tea wine's physicochemical and biochemical properties

Previously, *Majumder et al*. (2022a) analysed the physicochemical and biochemical properties of TW in comparison with control wine, where they highlighted the differences between the tea wine and the control wine sample and demonstrated the impact of the various groups of phytochemicals present in tea as substrate metabolites. The pH (one of the most important physicochemical properties of wine) was found to be very low in the TW compared to the control wine. Generally, a number of organic acids are produced during the fermentation of carbohydrates as common yeast metabolites following the glycolysis and further Kreb's cycle (as shown below in *Figure 4*), which increase the acidity of the fermented broth. Here, the GC-MS analysis revealed many such organic acid derivatives. The total peak area of such acid products may account for the amount or ratio of total organic acid content present in the tested wine samples. A total of 21.19% peak area in TW's chromatogram comprised various organic acid (carboxylates) derivatives (acetic acid, butanoic acid, succinic acid, levulinic acid, pyruvic acid, oxalic acid, etc.), while CW contained only 0.11%. TW also contained fatty acid products (5.36% total peak area) unlike CW, which might also influence the pH. Furthermore, qualitative and quantitative biochemical tests were carried out by *Majumder et al.* (2022a). Reportedly, total phenol content, terpenoids, and alkaloids were found to be comparatively high in TW, while the glycerol content was found to be higher in control wine compared to TW, aligning with this GC-MS result, as shown in *Table 1*. TW contains various bioactive metabolites belonging to phenol, terpenoids, and alkaloids, in contrast to CW, where approximately 96% of the total peak area was attributed to glycerol and its derivatives. Thus, the reported physicochemical and biochemical properties have been validated through this metabolite profiling. *Figure 3* exhibits a 3-D area chart showing the percentage shares of different groups

of metabolites based on chemotaxonomy and biosynthesis pathways. The graphs in *Figure 3* are symmetrical to the chromatograms of TW and CW, where the peak of sugar alcohol products in CW corresponds to glycerine present in it, unlike the TW rich in various phytochemical components.

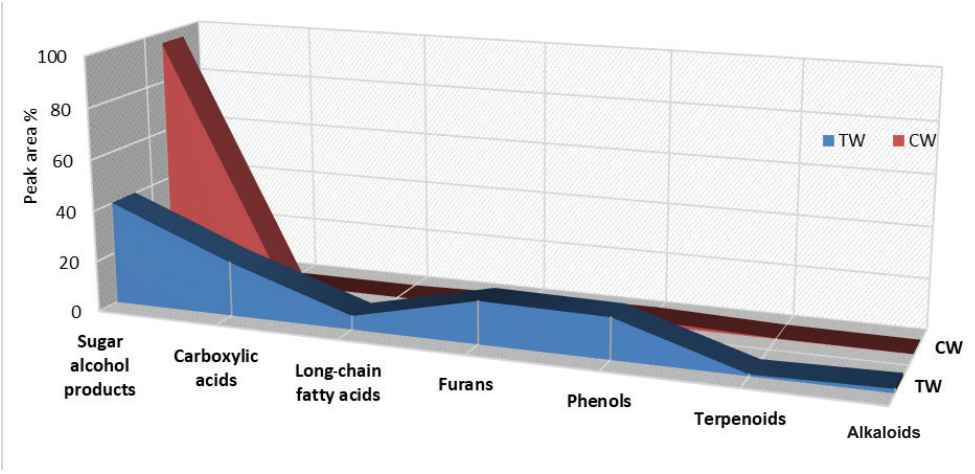


Figure 3. Peak area percentage shares by different classes of metabolites in the chromatograms

Medicinal properties of CTC-tea wine metabolites

The medicinal properties of exclusive TW metabolites have been described here under different sub-subheadings prepared aiming at different bioactivities, i.e. antioxidant, hepatoprotective and antidiabetic, antimicrobial and other medicinal properties, including anti-inflammatory, cardioprotective, anticancer, neuroprotective properties, etc. The reported medicinal properties of bioactive TW's metabolites are listed in *Table 2*.

Table 2. Bioactive compound of CTC-tea wine (TW) and reported medicinal properties

Name of the compound	Reported medicinal properties*
4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	Antioxidant, antimicrobial, anti-inflammatory, anti-diabetic, anti-proliferative, and hepatoprotective
Furfural	Antioxidant, hepatoprotective, antimicrobial

Name of the compound	Reported medicinal properties*
Palmitic acid derivatives	Antioxidant, anti-inflammatory, antimicrobial, cardioprotective (hypocholesterolaemia), anticancer properties
Levulinic acid	Antioxidant
Tyrosol	Antioxidant, hepatoprotective (anti-lipidperoxidation), antidiabetic, antimicrobial, anti-inflammatory
Furfuryl alcohol	Antioxidant, antimicrobial
1-hexadecanol	Antimicrobial (anti-staphylococcal)
Methylglyoxal or pyruvaldehyde	Antimicrobial, anticancer
Isosorbide dinitrate (sorbitol derivative)	Preventative against chest pain (angina), vasodilator, cardioprotective (treats achalasia, chronic painful diabetic neuropathy, ischemic cardiovascular diseases, congestive heart failure, oesophageal spasms, etc.)
Caffeine	Central nervous system stimulant, cardioprotective (induces diuresis), promotes secretion of gastric acid, alleviates migraine

Note: References are given in the text below.

Antioxidant compounds

Enhancement of food antioxidant properties during fermentation corresponds to the changes in physicochemical and biochemical attributes, which are interrelated with the metabolic actions of the employed starter – here: wine yeast *Saccharomyces cerevisiae*. Exclusive TW major compounds 4H-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- (15.58%) and furfural (4.99%) are reported as potential antioxidant molecules having several other biological activities (*Majumder et al.*, 2021). Antioxidant fatty acid- palmitic acid and its derivatives were detected in TW, which were previously reported as antioxidative fatty acids of red wine by *Yunoki et al.* (2004). *Yi* and *Kim* (1982) reported on the role of levulinic acid and furfural behind the antioxidant activity of wine. *Covas et al.* (2003) reported on the antioxidant activity of detected phenolic tyrosol. *Osada* and *Shibamoto* (2006) determined the antioxidant properties of furfuryl alcohol. Previously, TW was reported to exhibit significantly high antioxidant activity (*Majumder et al.*, 2022a) and, likewise, in this GC-MS analysis the sample was found to contain such antioxidative wine volatiles as 4H-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-, furfural, furfuryl alcohol, levulinic acid, palmitic acid, and tyrosol.

Hepatoprotective and antidiabetic compounds

A high lipid peroxidation inhibition property was reported for the sample TW (*Majumder et al.*, 2022a). The presence of the hepatoprotective compound tyrosol is the possible reason behind this result. *Kalaiselvan et al.* (2016) reported the attenuation of hepatic oxidative stress by hydroxytyrosol and tyrosol, which corresponds to the anti-lipidperoxidation activity of TW. *Chandramohan et al.* (2015) reported the antidiabetic activity of tyrosol, which is in line with the *in vitro* antidiabetic activity reported for TW (*Majumder et al.*, 2022a). Furfural has the potential to inhibit alcohol dehydrogenase, aldehyde dehydrogenase, and pyruvate dehydrogenase (*Modig et al.*, 2002), which could be useful in preventing poisoning from alcohols that metabolize into toxic products and also in preventing alcohol-induced inflammatory responses and in playing a role as a hepatoprotective agent.

Antimicrobial compounds

Antioxidant 4H-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- is a yeast secondary metabolite that possesses antifungal properties (*Majumder et al.*, 2021). *Togashi et al.* (2007) demonstrated the potential antibacterial activity of a TW metabolite, 1-hexadecanol, against *Staphylococcus aureus*. This also corresponds with our previous finding, where crude TW sample produced inhibition zone against *Staphylococcus aureus*. Potential antibacterial properties of detected furan derivatives – furfural and furfuryl alcohol – were also reported (*Chai et al.*, 2013; *Kalt* & *Cock*, 2014). Furfural has been reported as mutagenic against *Salmonella typhimurium* (*Zdzienicka et al.*, 1978). Methylglyoxal or pyruvaldehyde (detected as pyruvaldehyde dimethyl acetal) also exhibits antimicrobial activity (*Atrott* & *Henle*, 2009). Therefore, the presence of the antimicrobial compounds 4H-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-, furfural, furfuryl alcohol, tyrosol, pyruvaldehyde, and anti-staphylococcal 1-hexadecanol might be responsible for the reported antibacterial properties of TW against Gram-positive *Staphylococcus aureus* and *Bacillus subtilis*, as well as Gram-negative *Klebsiella pneumoniae* (*Majumder et al.*, 2022a).

Metabolites with other bioactivities

Overall, the detection of 4H-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- as major compound was an important finding of this research because this molecule can possess a wide range of bioactivities, including antimicrobial, anti-inflammatory, antidiabetic, antioxidant, anti-proliferative, and hepatoprotective properties (*Teoh & Don*, 2015; *Hameed et al.*, 2015; *Chakraborty et al.*, 2023), which could help the novel TW to show promising medicinal properties. The major fatty acid palmitic

acid and its derivatives also have anti-inflammatory, antioxidant, antimicrobial, cardioprotective (hypocholesterolaemia), and anticancer properties (*Majumder et al.*, 2021). Isosorbide dinitrate, a probable sugar-fermented product (sorbitol derivative), exhibits a range of medicinal properties to be celebrated as a life-saving drug. It plays a role as preventative against chest pain (angina), and it also functions as potential vasodilator. Further, it prevents anti-cardiovascular diseases, treats achalasia, chronic painful diabetic neuropathy, ischemic cardiovascular diseases, congestive heart failure, oesophageal spasms, etc. (https://go.drugbank.com/drugs/DB00883). The phenolic antioxidant metabolite tyrosol exhibits anti-inflammatory properties and exerts its beneficial effects against hypertension, atherosclerosis, coronary heart disease, chronic heart failure, insulin resistance, and obesity as well (*Karković Marković et al.*, 2019). Antimicrobial methylglyoxal or pyruvaldehyde has also been reported as an anticancer compound by *Talukdar et al.* (2009). The bioactivity of the signature tea-leaf compound caffeine as a central nervous system stimulant is well established and its presence in CTC-tea wine is one of the useful findings of this research. Caffeine induces diuresis, which shows positive effects on the cardiovascular system, and it also promotes the secretion of gastric acid and alleviates migraine as well (*Yang et al.*, 2010).

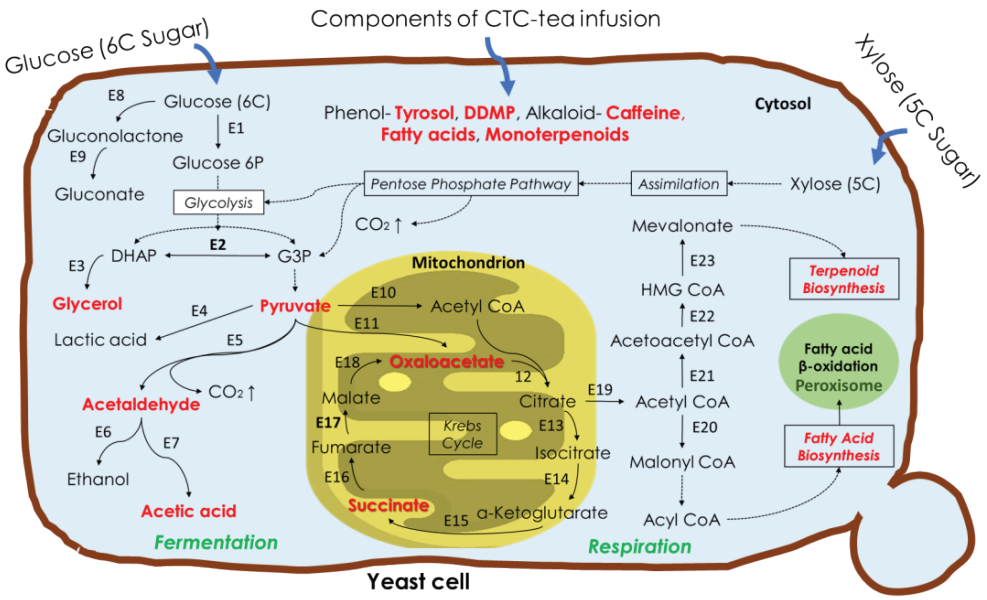
Flavour-imparting molecules of CTC-tea wine

According to The Good Scents Company database (http://www.thegoodscents company.com/), compounds like glycerine derivatives, acetic acid, pyruvaldehyde, furfural, furfuryl alcohol, levulinic acid, succinic acid, palmitic acid, etc. probably have a synergistic contribution towards the development of a sweet, caramel-like, astringent, slightly sour, and wine-like flavour as well as taste of the CTC-tea wine sample. The major compound 2,4-dihydroxy-2,5-dimethyl-3(2H)-furanone (6.23%) has been documented as an aroma-imparting component (potential fruity and wine-like aroma in wines) (*Chukwu et al.*, 2017). Butanoic acid, 2-ethyl-, methyl ester (2.31%) is also reported to exhibit wine-aroma-imparting properties (*Wei et al.*, 2019). Moreover, butanoic acid and furfural are reported as volatile aroma compounds of some famous Chinese liquors, which are probably considered as aging markers of some wines (*Xu et al.*, 2017). Butanoic acid and succinic acid are yeast metabolites and are reported as fermentation products (*Wilson et al.*, 2021) responsible for a distinct cidery flavour. So, TW is rich in both sugar alcohols and organic acids, and together these would produce a sweet and sour taste. (+-)-4-Amino-4,5-dihydro-2(3H)-furanone is a gamma-butyrolactone (major red wine aroma compound) derivative, a yeast metabolite that has been recognized for the unique flavour of wine and medicinal properties (*Vose et al.*, 2001; *Majumder et al.*, 2022b).

Biosynthesis pathways of bioactive compounds

Fermentation metabolomics depends on both substrate (here sugared CTC-tea infusion) and starter (here brewer's yeast or *Saccharomyces cerevisiae*). *Saccharomyces cerevisiae* is the principle agent for commercial wine fermentation that converts hexose and pentose sugars to fermented products like ethanol, glycerol, carbon dioxide, and a variety of compounds: alcohols, esters, aldehydes, and acids, which contribute to the sensory attributes of the beverage (*Viljoen*, 2006). The fundamental yeast metabolic pathway has been given in *Figure 4*, which is self-explanatory showing the enzymatic steps responsible for the glycolysis of six-carbon sugars, the assimilation and pentose phosphate pathway for five-carbon sugars, the production of ethanol, glycerol, and acetic acid (major fermented beverage composition), the Krebs cycle, fatty acid biosynthesis, and beta-oxidation, terpenoid biosynthesis, etc. In this regard, the Yeast Metabolome Database (http://www.ymdb.ca/) and the KEGG pathway database (https://www.genome.jp/kegg/pathway.html) were accessed, and numerous research papers on *Saccharomyces* yeasts were reviewed to design these possible pathways involved in the biosynthesis of TW metabolites (*Figure 4*).

During yeast fermentation, hexose (i.e. glucose) and pentose (i.e., xylose) sugars present in the substrate or broth entered the glycolysis pathway and were further converted into dihydroxyacetone-phosphate (DHAP) and glyceraldehyde-3-phosphate. Enzyme glycerol-3-phosphate dehydrogenase produced glycerol (the major control wine metabolite detected in this research) from DHAP, while glyceraldehyde-3-phosphate was converted into pyruvic acid, which was the precursor of ethanol, acetaldehyde, and organic acids (lactic acid, acetic acid, succinic acid, etc.) (*Figure 4*). Pyruvate decarboxylase converted pyruvic acid into acetaldehyde and carbon dioxide. Acetaldehyde was further converted into ethanol and acetic acid, the major fermented beverage metabolites (*Figure 4*). On the other hand, mitochondrial pyruvate dehydrogenase complex and pyruvate carboxylase biosynthesised other organic acids and acetyl CoA, which was an intermediate to terpenoids' and fatty acids' biosynthesis and further to the beta oxidation of fatty acids in peroxisome following the respiration process (*Figure 4*). Moreover, phenolic-tyrosol (*Liu et al.*, 2023), 4H-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- (*Ma et al.*, 2023), purine alkaloid caffeine (*Majumder et al.*, 2020), detected fatty acids and terpenoids (*Wei et al.,* 2023) could be possibly introduced into tea wine product as tea infusion components, being reported as secondary metabolites of tea.



Notes: Metabolites written in red were involved in TW's metabolome.
(DHAP: dihydroxyacetone phosphate; G3P: glyceraldehyde-3-phosphate; DDMP: 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-)
[E1: Hexokinase; E2: Triose-phosphate isomerase; E3: Glycerol-3-phosphate dehydrogenase; E4: Lactate dehydrogenase; E5: Pyruvate decarboxylase; E6: Alcohol dehydrogenase; E7: Acetaldehyde dehydrogenase; E8: Glucose oxidase; E9: Lactonase; E10: Pyruvate dehydrogenase complex; E11: Pyruvate carboxylase; E12: Citrate synthase; E13: Aconitase; E14: Isocitrate dehydrogenase; E15: Alpha-Ketoglutarate dehydrogenase/ Succinyl-CoA synthetase; E16: Succinate dehydrogenase; E17: Fumarase; E18: Malate dehydrogenase; E19: ATP citrate (pro-S)-lyase / ATP citrate synthase; E20: Acetyl-CoA carboxylase; E21: Acetyl-CoA C-acetyltransferase; E22: Hydroxymethylglutaryl-CoA (HMG CoA) synthase; E23: HMG-CoA reductase]

Figure 4. The proposed yeast metabolic pathway to understand TW's metabolomics

Glycerine was detected as the major compound in both CW (86.43%) and TW (26.6%). Glycerine is produced by fermenting yeast during beverage fermentation when ethanol and CO_2 (main by-products) formation stops. Yeast metabolism is divided into two major pathways: firstly where ethanol, acetic acid, and carbon dioxide are produced and secondly where glycerol is produced. In the case of fermented alcoholic beverages, both ethanol and glycerol are produced by fermentation of sugars (*Majumder et al.*, 2022d). Glycerol, 1-acetate (11.61%) was a glycerol derivative in TW sample, where the fermented product glycerol was esterified with another common sugar fermented product: acetic acid.

The plant cuticle is composed of cutin (a polymer of cross-esterified hydroxy-fatty acids) and a mixture of long-chain hydrocarbons (long-chain fatty acids,

fatty alcohols, alkanes, etc.), known collectively as waxes. Fatty acid derivatives, i.e. octyl octanoate, tridecyl acrylate; octanoic acid, 4-pentadecyl ester; palmitic acid derivatives; furfuryl heptanoate; the phenolic compound tyrosol, a strong antioxidant of tea (*Liu et al.,* 2023), monoterpenoids, i.e. myrtenol and trans-ascaridol glycol, etc. are common plant metabolites which might be present as tea/substrate metabolites in the fermented broth. However, yeast fermentation can also be the reason behind the production of fatty acids as designed on the pathway (*Figure 4*). Previously, caffeine and steroids were qualitatively determined in this particular TW sample (*Majumder et al.*, 2022a). Therefore, the detection of caffeine in its metabolite profile is valid.

Furfural and other furan derivatives detected in TW are reported as metabolites of fermenting microbes and common aroma components of fermented beverages as wine, brandy, whiskey, rum, etc. (*Majumder et al.*, 2021). Furfuryl alcohol occurs mainly due to the enzymatic or chemical reduction of furfural during the aging of a wine (*Majumder et al.*, 2021) and is a major volatile compound of beer (*Wei et al.*, 2001). Butanoic and succinic acids are yeast metabolites; therefore, their biosynthesis in tea wine is valid. Succinic acid is also the precursor of levulinic acid; these metabolites are reported as glucose/fructose fermentation products and are mainly found in aged wine and beer samples (*Majumder et al.*, 2021).

The major bioactive compounds of TW, i.e. 4H-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- or hydroxydihydromaltol (15.58%) and furfural derivatives, are strong antioxidants, Maillard reaction products of sugar (formed due to the thermal dehydration of sugars) found in fermented beverages, which might be derived from sugar fermentation or storage (*Majumder et al.*, 2021). Preparation of CTC-tea infusion (fermentation substrate) was done at high temperature (98 ± 1 °C) that might possibly induce Maillard reaction (*Kchaou et al.*, 2019). *Idowu et al.* (2017) and *Ma et al.* (2023) reported the occurrence of 4H-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- in different green teas and sun-dried Pu-erh tea. Previously, 4H-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- was reported as a sugar derivative, which is a possible derivative of a flavonoid biosynthesised during fermentation by yeast (*Majumder et al.*, 2021) because it is reported as a compound with flavonoid fraction and as a fungal secondary metabolite having antifungal properties (*Teoh & Don*, 2015). The production of furfural derivatives and levulinic acid has been reported from a rich carbohydrate source, i.e. lignocellulosic biomass (*Li et al.*, 2019). Therefore, furan derivatives (furfural, furfuryl alcohol, and furfuryl heptanoate) and levulinic acid may be derived from sugar during the fermentation process. *Velaga & Peela* (2022) showed the thermal decomposition of furfural and reported the green synthesis of levulinic acid. The high temperature of sugared black tea infusion (substrate) (98 ± 1 °C) could induce the production of levulinic acid. *Maharramov et al.* (2020) reported the production of furfurals in processed Azerbaijan Tea. *Parveen et al.* (2023) also identified furfurals as major compounds in black tea produced by

Camellia sinensis and *Camellia assamica*. *Maldonado et al.* (2012) demonstrated the acid-catalysed conversion of furfural alcohol to levulinic acid. Therefore, inside an acidic fermenting broth (TW) rich in organic acids, the detection of furfural and furfuryl alcohol alongside levulinic acid is also valid, and they belong to the same pathway. *Wang et al.* (2022) reported the dehydration of monosaccharides, such as fructose and glucose, to produce levulinic acid. Hence, infusion-induced non-enzymatic physicochemical reactions (like the Maillard reaction) and enzymatic biochemical changes due to fermentation or storage both steered the production of these components in TW. Further, working with tea-flower wine, *Majumder et al.* (2021) reported antioxidant agent levulinic acid and its precursor succinic acid (also detected in TW) as products of glucose fermentation, which are mainly found in aged wine and beer samples. Pyruvaldehyde dimethyl acetal is a sugar-fermented product either biosynthesised from pyruvic acid (also detected in TW) and acetaldehyde during alcoholic fermentation or produced as a result of the oxidative degradation of dihydroxyacetone (or glycerone, the precursor of glycerine) (*Lip et al.*, 2013) during glycerine production. Caffeine detected in TW occurred as tea metabolite, as CTC-tea infusion was the fermentation substrate. Glycerine; glycerol, 1-acetate; furfural, furfuryl alcohol, 2,4-dihydroxy-2,5-dimethyl-3(2H)-furan-3-one; organic acids (butanoic acid, succinic acid, levulinic acid, cyclobutanecarboxylic acid, pyruvic acid, acetic acid and oxalic acid); 4H-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- or hydroxydihydromaltol; 3-methyl-2(5H)-furanone, etc. belonging to sugar derivatives metabolome are directly associated with carbohydrate fermentation, while, through detection of compounds like phenolic- tyrosol; alkaloid- caffeine; flavonoid fraction 4H-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-; fatty acid-palmitic acid; fatty acid derivatives, i.e. octyl octanoate, tridecyl acrylate; octanoic acid, 4-pentadecyl ester; palmitic acid derivatives; heptanoate; monoterpenoids-myrtenol and trans-ascaridol glycol, etc., the effect of CTC-tea in the fermentation has also been reflected. Altogether, the metabolites – whether biosynthesised or generated through non-enzymatic reactions – derived from sugar and tea fermentation or directly from the infusion have validated the bioactive potential and acceptability of this tea wine.

4. Conclusions

A major part of our regular diet, particularly green vegetables and beverages, such as tea and wine, serves as substantial sources of antioxidants. Given their richness in bioactive components, fusion products like tea wine hold the potential to align seamlessly with consumer acceptability. As the fields of wine research and tea science continue to evolve, the findings of this study can serve as a foundation for future investigations and innovations in the realm of product development

from tea. Researchers, policymakers, and industry professionals are encouraged to continue exploring innovative solutions based on tea, particularly focusing on CTC tea. Despite its relatively lower economic value compared to premium orthodox black tea and green tea, CTC tea exhibits substantial production potential and has a high demand in both the national and the global market. In the Indian scenario, some portion of manufactured tea (mostly CTC tea) remains unsold, and the brewing industry may utilize this unsold tea for its value-added form, i.e. tea wine. These untapped possibilities have the potential to significantly boost the growth of the tea industry. Moreover, the physicochemical, biochemical, and medicinal properties of TW have been successfully evaluated through this follow-up research work on GC-MS-based metabolomics. Alterations in the polyphenolic profile of black tea (i.e. theaflavins and thearubigins) should be analysed by using HPLC/LC-MS techniques. Further sophisticated instrumentations, scientific research trials, and value additions are needed to uphold the acceptability of this wine.

Authors' contribution

S. M. and M. B. designed the research. S. M., A. G., and S. C. carried out the experiment. S. M. analysed the data and wrote the draft manuscript. M. B. and S. M. revised the manuscript. M. B. supervised the research work.

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Bioactive composition, antioxidant activities, and health benefits of selected tropical horticultural fruit waste

O. M. Oni*
e-mail: yemisioniconsult@gmail.com

O. T. Ademoyegun
e-mail: femtopyankee@gmail.com

D. O. Raphael
e-mail: olibest133@gmail.com

T. M. Fasuan
e-mail: titilope.olagunju@yahoo.com

National Horticultural Research Institute, Ibadan, Nigeria

Abstract. This study sought to evaluate the potential benefits of tropical horticultural fruit waste, its bioactive composition, and possible reasons for its underutilization and to provide comparative knowledge that could be adopted for pharmaceutical, culinary, and therapeutic purposes. Guava seed was found to be richer in β-sitosterol as compared to extracts from the seeds of pawpaw, soursop, passion fruit, pumpkin, mango, grape, tomato, and orange. The sweetness-inducing effect of miraculin from "miracle fruit" and its effect on TAS1R3 and TAS1R2 taste receptors were evaluated. Upon a critical study of existing research data, this study has found the identification of bioactive composition and active ingredient as insufficient in boosting utilization of fruit waste and faulted the relatively low adoption of research findings by consumers and food processing to be due to limited research and awareness on possible methods of processing horticultural waste into readily available, commercializable forms.

Keywords and phrases: mechanism, food enrichment, oxidative stress, therapeutic value, food loss and waste

Introduction

Tropical horticultural fruits typically refer to fruits grown in equatorial or warm-climate areas. They are considered good sources of essential minerals, dietary fibres, and bioactive compounds with varying health benefits. In spite of potential nutritional and therapeutic benefits, parts of fruit, such as peels,

rinds, seeds, pomace, and core, are considered waste by most consumers and processing industries despite accounting for a significant portion of the entire fruit weight, with waste generated from fruits such as pineapple and orange accounting for approximately 40% and 55% (w/w) respectively (*Zhang et al.*, 2020). Underutilization and poor food waste management practices have greatly contributed to the growing challenge of environmental pollution, which generates an equivalent of 3.3 billion tons of greenhouse gases annually (*FAO*, 2022).

Global demand for fruit continues to increase due to dietary recommendations. Fruits are characterized by a relatively short shelf life as compared to tuber crops. As such, waste from tropical horticultural fruits poses not only environmental challenges but economic complications as well (*Martínez-Inda et al.*, 2023). In an attempt to address increased cases of undernutrition, particularly in developing communities, food insecurity, and reduced agricultural yield due to climate change, researchers and agro-allied stakeholders alike have sought means of value adding for horticultural waste in order to maximize their nutritional benefits, reduce food waste, and increase food availability. By-products of citrus fruits (seed, pomace, and wastewater) account for 50–60% of the weight of raw fruit after processing or household consumption (*Panwar et al.*, 2021).

This prompted the need for further research into bioactive and proximate compositions as well as suitable methods for reprocessing various tropical horticultural food waste. Tropical fruits such as mango, grapefruit, guava, and orange are rich in vitamin C, a powerful antioxidant known to inhibit free radicals and oxidative stress. Other examples of identified health benefits of horticultural fruit wastes include: anti-inflammatory and anti-proliferation of cancer cells by avocado seed (*Vo et al.*, 2019; *Alkhalaf et al.*, 2019); high dietary fibre in guava seeds, which facilitates improved bowel health and healthy weight loss (*Uchôa-Thomas et al.*, 2014); inhibitory activities of sweet orange *Citrus sinensis* seeds against *E. coli*, *S. aureus*, *Pseudomonas aeruginosa*, and *Salmonella spp.* bacterial (*Oikeh et al.*, 2020).

Value addition for tropical horticultural fruit waste as a primary or secondary raw material in cosmetics, industrial processing, food enrichment, or fortification cannot be achieved without the knowledge of its proximate and bioactive composition. This information helps in determining the most appropriate method of utilization (*Raj & Masih*, 2014). This study seeks to evaluate the potential health benefits of tropical horticultural fruit waste, its bioactive composition, and possible reasons for its underutilization.

2. The chemical composition of selected horticultural fruit waste

The health benefits of fruits cannot be overemphasized, as they serve as important sources of much-needed vitamins, minerals, and bioactive compounds of health-promoting potentials. The quantity of these substances in fruits may vary based on varieties, species, and in some cases the climatic condition of places where they are cultivated. An existing misconception pertaining to health benefits attainable in tropical fruits is continually fuelled by inadequate or misinformation from media platforms, medical practitioners, and nutritionists who often recommend fruits only based on health benefits attainable within the mesocarp, neglecting health-promoting potentials accessible in other parts of the fruit. Examples of this include the predominance of β-sitosterol in the seeds of guava, higher phenolic and flavonoid contents identified in the peels and seed of avocado fruit as compared to its pulp.

Findings from existing literature, as shown in *Table 1*, highlight the concentration of polyphenols and the antioxidant capacities identified in selected tropical horticultural fruit waste. This information is vital for appraising health-promoting potentials and possible means of utilizing the selected fruit waste. High flavonoid and phenolic content often results in increased antioxidant capacity. The presence of different active ingredients identified in different waste parts (*Table 3*) are largely responsible for the observed health benefits of waste parts, as shown in *Table 2*.

2.1. Pineapple

The proximate composition of pineapple peels comprises moisture (82.7%), ash (5.0%), total lipids (1.1%), crude protein (8.8%), and crude fibre (16.3%) on a wet basis (*Morais et al.*, 2017). The pineapple peel is a good source of ascorbic acid and ferulic acid and possesses anti-inflammatory, antioxidant, and antimicrobial properties (*Sah et al.*, 2016; *Lubaina et al.*, 2019). In Nigeria and some parts of Africa, pineapple peel is boiled with roselle calyces to improve its sensory and nutritional attributes. It can also be applied as a condiment in the production of culinary meals (*Wu & Shiau*, 2015). Major phenolic compounds identified in pineapple peels include epicatechin, gallic acid, catechin, and ferulic acid (*Li* et al., 2014). Findings by *Zhang et al.* (2020) indicate fermentation to be an effective means of improving the phenolic and flavonoid content of pineapple peel. The high dietary fibre content of pulverized pineapple peel can also be adopted as an effective means of improving bowel movement and reducing the chances of overfeeding.

2.2. Miracle fruit

Miracle fruit contains anthocyanin, a substance known to reduce or prevent high blood pressure by inhibiting the angiotensin-converting enzyme (ACE) and thereby preventing vasoconstriction (*Raphael et al.*, 2023). The antioxidant nature of anthocyanin also makes miracle fruit an effective means of treating oxidative stress and preventing free-related illness. Miracle fruit contains the uncommon protein "miraculin", which can be used to activate sweet taste receptors in patients following chemotherapy.

Table 1. The phenolic, flavonoid, and antioxidant capacity of selected tropical horticultural fruit waste

Fruit	Parts	Phenolic	Flavonoid	Antioxidant	Reference
Orange (*Citrus sinensis*)	Peel	21.31 mg GAE/g	1.08 mg QE/g	4.79 mg AAE/g	(Suleria *et al.*, 2020)
		35.6 mg GAE/g	83.3 mg CE/g	70.5%	(Sir Elkhatim *et al.*, 2018)
Miracle fruit (*Synsepalum dulcificum*)	Seed	18.55 mg/g	0.88 µg/g	16.94 µmol/g	(Inglett & Chen, 2011)
	Skin	59.54mg/g	5.27 µg/g	27.15 µmol/g	(Inglett & Chen, 2011)
Grapefruit (*Citrus paradisi*)	Peel	27.22 mg GAE/g	0.82 mg QE/g	9.17 mg AAE/g	(Suleria *et al.*, 2020)
		77.30 mg GAE/g	80.8 mg CE/g	76.4%	(Sir Elkhatim *et al.*, 2018)
African star apple (*Ghrysophyllum albidum G.*)	Seed	279.79 mg GAE/100g	116.68 mg CAT/100g	50.67%	(Oluwatoyin *et al.*, 2017)
	Peel	308.30 GAE/100g	160.21 mg CAT/100g	40.14%	(Oluwatoyin *et al.*, 2017)
Pineapple (*Ananas comosus*)	Peel	7.83 mg GAE/g	1.47 mg QE/g	1.30 mg AAE/g	(Suleria *et al.*, 2020)
		11.1 mg GAE/g	3.86 mg QE/g	13.63 µmol TE/g	(Jatav *et al.*, 2022)
Guava (*Psidium guajava*)	Seed	973.80 mg/100g dw	270.3 mg/100g dw	63.74%	(El Anany, 2015)
	Peel	39.65 mg GAE/g dw	19.72 mg RE/g dw	264.3 µmol TE/g dw	(Liu *et al.*, 2018)

Fruit	Parts	Phenolic	Flavonoid	Antioxidant	Reference
Pawpaw (*Carica papaya*)	Seed	9.61 mg GAE/g	0.36 mg QE/g	4.3 mg TrE/g	(Gaye *et al.*, 2019)
	Peel	3.13 mg GAE/g	1.06 mg QE/g	1.33 mg AAE/g	(Suleria *et al.*, 2020)
		15.53 mg GAE/g	0.23 mg QE/g	1.96 mg TrE/g	(Gaye *et al.*, 2019)
Banana (*Musa spp.*)	Peel	6.13 mg GAE/g	1.32 mg QE/g	1.20 mg AAE/g	(Suleria *et al.*, 2020)
		22.95 mg GAE/g	13.68 mg GAE/g	79.03%	(Islam *et al.*, 2023)
Avocado (*Persea americana*)	Peel	123.57 mg GAE L-1	14.09 mg QE L-1	1957.24 µmol TE L-1	(Rotta *et al.*, 2015)
		18.79 mg GAE/g	1.24 mg QE/g	8.67 mg AAE/g	(Suleria *et al.*, 2020)
	Seed	8.72 mg GAE/100g	1.721 mg QE/100g	72.65%	(Siol & Sadowska, 2023)
Mango (*Magnifera indica*)	Peel	27.51 mg GAE/g	1.75 mg QE/g	8.67 mg AAE/g	(Suleria *et al.*, 2020)

Notes: GAE = gallic acid equivalent; QE = quercetin equivalent; AAE = ascorbic acid equivalent; TrE = trelox equivalent; CE =catechin equivalent.

Table 2. Common names of selected tropical horticultural waste and health benefits from waste part(s)

Fruit	Other name(s)	Parts	Health benefits	Reference
Orange	Osan, mchungwa	Peel	Anti-inflammatory, antioxidant, weight loss	(Chen *et al.*, 2011)
Miracle fruit	Miracle berry	Seed	Cardiovascular disease treatment, anti-diabetic, antioxidant	(Huang *et al.*, 2023)
Grapefruit	Pamplemousse	Peel	Antihypertensive, antioxidant, anti-diabetes	(Ademosun *et al.*, 2015; Oboh & Ademosun, 2011)
African star apple	Agbalumo, udara	Seed	Treatment of vaginal and dermatological infections	(Asare *et al.*, 2015)
		Peel	Antimicrobial, antioxidant	(Okoli & Okere, 2010)
Pineapple	Ananas	Peel	Antioxidant, antimicrobial, anti-inflammatory	(Sah *et al.*, 2016)

Fruit	Other name(s)	Parts	Health benefits	Reference
Lemon	Limau	Peel	Anti-inflammatory, anti-tumour, antioxidant, anticancer, antimicrobial	(Wang *et al.*, 2021)
Guava	Guayava, goiaba	Seed	Antimicrobial, anti-inflammatory, anti-cancer	(El Anany, 2015)
		Peel	Hypoglycaemic, antioxidant, weight loss	(Vijaya Anand *et al.*, 2019)
Pawpaw	Papai, ibepe	Seed	Anti-diabetes, anti-hypercholesterolemia, antioxidant, weight loss	(Saba, 2022)
		Peel	Antioxidant, anti-inflammatory	(Dada *et al.*, 2016)
Banana	Ogede, mgomba	Peel	Antioxidant, anti-inflammatory	(Azarudeen & Nithya, 2021)
Avocado	Alligator peer	Seed	Anti-hyperglycaemic, anti-hypertensive, antioxidant	(Bangar *et al.*, 2022)
		Peel	Antitumor, antioxidant, antidiabetic, anti-ageing	(Akan, 2021)
Mango	Mangwaro, mangues	Peel	Antioxidant, anti-cancer	(Ajila *et al.*, 2008)

Table 3. Bioactive compounds identified in selected horticultural wastes

Fruit	Botanical name	Family	Parts	Bioactive ingredients	Reference
Orange	*Citrus sinensis*	*Rutaceae*	Peel	Limonoid, carotenoids, ascorbic acid, protocatechuic acid, hesperidin, Catechin	(Montero-Calderon *et al.*, 2019)
Grapefruit	*Citrus paradise*	*Rutaceae*	Peel	Hesperidin, hesperetin, naringin, flavones, phenols	(Castro-Vazquez *et al.*, 2016)
Pineapple	*Ananas comosus*	*Bromeliaceae*	Peel	Ferulic acid, gallic acid, Catechin, epicatechin, phenols, malic acid, ascorbic acid	(Meena *et al.*, 2022; Rivera *et al.*, 2023)
Lemon	*Citrus limon*	*Rutaceae*	Peel	Terpenes, limonoid, pectin, flavonoid, hesperidin	(Saini *et al.*, 2022)

Fruit	Botanical name	Family	Parts	Bioactive ingredients	Reference
Guava	*Psidium guajava*	*Myrtaceae*	Seed	Oleic acid, linoleic acid	(Uchôa-Thomas *et al.*, 2014)
			Peel	Lycopene, phenols, myricetin, apigenin	(Bertagnolli *et al.*, 2014; Vijaya Anand *et al.*, 2019)
Pawpaw	*Carica papaya*	*Caricaceae*	Seed	Vanillic acid, ferulic acid, glycosides, ferulic acid	(Rodrigues *et al.*, 2019)
			Peel	Papain, chymopapain, pantothenic acid	(Balavijaya-lakshmi & Ramalakshmi, 2017)
Banana	*Musa acuminate*	*Musaceae*	Peel	Catecholamines, phlobatannins, triterpenes	(Hikal *et al.*, 2022)
Avocado	*Persea americana*	*Lauraceae*	Seed	Procyanidins, phenols, ascorbic acid	(Bangar *et al.*, 2022)
			Peel	Rutin, epicatechin, quercetin, chlorogenic acid	(Martínez-Gutiérrez, 2023)
Mango	*Magnifera indica*	*Anacardiaceae*	Peel	Magniferin, benzoic acid, polyphenols, Catechin	(Coelho *et al.*, 2019)

Miraculin induces sweetness by interacting with TAS1R3 and TAS1R2 sweet taste receptors at the gastrointestinal and oral cavity levels, stimulating them under acidic conditions (*Gómez de Cedrón* et al., 2020). This makes miracle fruit an ideal and low-calorie alternative to sugar in sour food or drinks. Miracle fruit seed was found to be typically high in potassium and also a potential treatment for Alzheimer's disease. Other identified bioactive components include phytosterols, lignins, tritepenoids, phenolic acids, and N-cis-caffeoyltyrmine (*Akinmoladun et al.*, 2020; *Huang et al.*, 2023).

2.3. African star apple

African star apple fruit, also known as "agbalumo" or "udara" in Nigeria, is grown predominantly in the tropical parts of Africa. Like the case of miracle fruit, there are limited research studies on the health benefits and alternative methods of utilizing this indigenous fruit. Seeds of African star apples are rich in minerals,

such as calcium, magnesium, sodium, and potassium, as compared to African star apple peels (*Abolaji & Henry*, 2015), with a 11.6–23.80% oil yield (*Anang et al.*, 2019; *Omeje et al.*, 2019). Fatty acids extracted from African star apple seed include undecylenic acid and oleic acid, with studies proving the anti-inflammatory properties of undecylenic acid via the inhibition of cyclooxygenase activity (*Van der Steen & Stevens*, 2009), while oleic acid, a mono-unsaturated omega-9 fatty acid, reduces cholesterol and improves cardiovascular health (*Lopez-Huertas*, 2010).

The seed of the African star apple is useful in the treatment of vaginal and dermatological infections (*Asare et al.*, 2015), while its peel possesses antimicrobial and antioxidant properties (*Okoli & Okere*, 2010).

2.4. Orange

Parts of citrus fruit, such as peels, seeds, and rinds, are considered waste and are often discarded despite their therapeutic and nutraceutical values (*Sir Elkhatim et al.*, 2018) and account for half the mass of the entire fruit. Antioxidant and anti-diabetic properties were also observed in the peels of grapefruit (*Ademosun et al.*, 2015; *Oboh & Ademosun*, 2011), while the antifungal activity of orange peel was observed against *Histoplasma capsulatum* and *Aspergillus niger*. Orange peels and seeds showed varying degrees of inhibitory activities against gram-negative and gram-positive bacteria such as *Pseudomonas fluorescens, Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli, Proteus spp., Enterococcus faecalis*, and *Salmonella choleraesius* (*Naqvi et al.*, 2021; *Oikeh et al.*, 2020).

Orange peels are also known for their anti-inflammatory, antioxidant, and weight loss properties (*Chen et al.*, 2011; *Chen et al.*, 2017). This was in accordance with findings by *Gosslau et al.* (2018), who attributed reduced inflammation observed in rats to the activation of the NF_KB signalling pathway, resulting in increased production of chemokines and induced cell apoptosis. Flavone glycoside (hesperidin), a naturally occurring bioactive compound found in citrus peels, is used in the treatment of central nervous disorders and oxidative stress-related ailments (*Kim et al.*, 2019).

2.5. Banana

Banana peels account for 36.6% of the mass of banana fruit (*Soltani et al.*, 2011), which equates to 52.70 million metric tons of wasted banana peel annually (*Dadrasnia et al.*, 2020). Peels of banana (*Musa spp.*) are rich in minerals and essential fatty acids such as oleic acid, stearic acid, palmitic acid, and linolenic acid (*Mohd Zaini et al.*, 2022), potassium, iron, zinc, magnesium, calcium, carbohydrate, and dietary fibre (*Islam et al.*, 2023; *Kabir et al.*, 2021).

Research conducted by *Sundaram et al.* (2011) indicates higher phenolic and antioxidant activities in unripe banana peel as compared to ripe peel; their findings were in accordance with deductions by *Fatemeh et al.* (2012). Polyphenolic compounds identified in banana peels include rutin, kaempferol-3-rutinoside, triferuloyl-dihexose, quercetin-3-rutinoside, myricetin-deoxyhexose-hexoside, isoquercitrin, p-coumaric acid, and sinapic acid (*Mohd Zaini et al.*, 2022; *Vu* et al., 2018), which can be utilized for their antioxidant, antimicrobial, and anti-inflammatory properties (*Azarudeen & Nithya*, 2021; *Naksing et al.*, 2021).

2.6. Pawpaw

The anti-oxidative nature of pawpaw seed makes it a viable option for protecting non-tumorigenic cells (HepG2) from oxidative stress (*Salla et al.*, 2016). Seeds of pawpaw were also found to contain lycopene, a substance known for its highly reactive nature against free radicals (*Kumar & Devi*, 2022). Utilization of unripe pawpaw is highly encouraged, as studies indicate a higher concentration of potassium, calcium, sodium, magnesium, and phosphorus as compared to waste generated from ripe pawpaw (*Chukwuka et al.*, 2013). *Carica papaya* peels show antioxidant and anti-inflammatory properties (*Dada et al.*, 2016), while the seeds are also known for their anti-diabetes, anti-hypercholesterolemia, antioxidant, and weight loss properties (*Saba*, 2022). Utilization of pawpaw seed and peels is encouraged despite the identified presence of hydrogen cyanide (an anti-nutrient), as research findings indicate a significantly lower concentration of the aforementioned anti-nutrient as compared to its recommended permissible limit consumption (*Egbuonu*, 2017). As such, it can be used in the fortification of foods to improve flavour, colour, or therapeutic benefits (*Omar et al.*, 2020).

2.7. Avocado

Peels and seeds of avocado are increasingly utilized in tea production (*Rotta et al.*, 2015), cosmetics (*Ferreira et al.*, 2022), and baking flour alternatives (*Mahawan et al.*, 2015; *Novelina et al.*, 2022). This, in large part, could be attributed to the increased awareness of its numerous health benefits.

Avocado seed possesses antioxidant, anti-cancer, anti-inflammatory, and anti-proliferative properties (*Alkhalaf et al.*, 2019; *Vo et al.*, 2019), while anti-tumour, antioxidant, antidiabetic, and anti-aging properties have been observed in avocado peels (*Akan*, 2021). The presence of mono-unsaturated fatty acids in its seed and peels also highlights its anti-hypercholesterolemia potential. Identified bioactive compounds in the peels and seeds of avocado fruit include benzoic acid, catechin, pyrogallol, chlorogenic acid, rutin, and protocatechuic acid (*Zaki et al.*, 2020),

tocopherols, carotenoids, and procyanidins (*Concepción-Brindis et al.*, 2022; *Rojas-García et al.*, 2022).

2.8. Mango

Mango peels are a good source of phenols, flavonoids, carotenoids, iron, and zinc (*Baddi et al.*, 2015; *Marçal & Pintado*, 2021) and are considered suitable for the production of probiotic foods due to the presence of soluble fibre in mango peels. Studies indicate the anti-asthmatic properties of magniferin (a major bioactive compound identified in mango peel) via the attenuation of Th1/Th2 cell ratio and the activation of T-cell-specific transcription factor GATA-3 (*Imran et al.*, 2017). Mango peels can be dried using various drying techniques, milled, then applied as a food supplement, primary or secondary raw material for baking (*Ajila et al.*, 2008), or in other forms of food processing (*Ajila et al.*, 2010).

2.9. Guava

Findings by *da Silva & Jorge* (2017) indicate a significantly higher concentration of phytosterol (β-sitosterol) in guava seed extract as compared to extracts from seeds of pawpaw, tomato, soursop, orange, grape, passion fruit, mango, and pumpkin. β-sitosterol decreases cholesterol levels in the blood by reducing cholesterol absorption from the diet, thus resulting in improved cardiovascular health (*Saeidnia et al.*, 2014). β-sitosterol identified in guava seed can also be utilized for its neuroprotective, angiogenic, antimicrobial, and antioxidant effects (*Novotny et al.*, 2017). Hypoglycaemic, antioxidant, and weight-loss-inducing effects were observed in guava peels (*Vijaya Anand et al.*, 2019).

Conclusions

Health benefits, bioactive composition, and reaction mechanisms of selected tropically generated fruit waste were evaluated. Anti-tumour, anti-inflammatory, antioxidant, anti-diabetic, anti-microbial, and anti-hypertensive activities identified in different fruit wastes served as significant indicators of their relatively untapped potential. However, during this review, limited advances pertaining to methods of possible utilization of proximate compositions and bioactive components of fruit-generated waste were observed, which could in part be faulted as a key reason for its underutilization. This was evident in the research conducted on African star apples and miracle fruits, for which limited research data was found.

The use of tropical horticultural fruit waste for its wide range of identified nutraceutical and therapeutic values is hereby recommended. The quantity of

horticultural fruit waste applicable during food processing or value addition exists at the user's discretion, as no evidence of detrimental effects was identified during this study.

There is need for more research studies, as limited data exists on methods of processing tropical fruit waste into storable and commercializable forms. This, in some parts, would explain the observed hesitation by food-processing industries in incorporating parts of fruit considered waste into their production process or their utilization in the production of entirely new products. There is a need for increased commitment to applied research, as this would offer a replicable pathway for an adoptable method of safely processing horticultural fruit waste.

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Technological aspects of lactic acid bacteria originated from artisanal cheeses

É. Laslo
e-mail: lasloeva@uni.sapientia.ro
ORCID:
https://orcid.org/0000-0003-0743-914X

É. György
e-mail: gyorgyeva@uni.sapientia.ro
ORCID:
https://orcid.org/0000-0001-7998-0432

A.-M. Szabó
e-mail: anna.keresztes@kosiskola.ro

Sapientia Hungarian University of Transylvania, Cluj-Napoca,
Faculty of Economics, Socio-Human Sciences and Engineering,
Department of Food Science, RO-530104 Miercurea Ciuc, 1 Libertății Sq.

Abstract. Well-characterized, genetically stable starter cultures are used to produce safe fermented dairy products of consistent quality. Lactic acid bacteria play several roles in cheese production. The lactic acid produced influences the firmness of the curd, the yield, and the rheological properties of the cheese. Starter cultures contribute to the formation of flavour and aroma compounds in the product.

The aim of the research is to select and determine the technological aspects of lactic acid bacteria isolated from fresh traditional cheese varieties, which could potentially be used as cheese starter. The 13 strains of the more than 50 lactic acid bacteria studied showed different proteolytic activities and moderate acidifier capacity, contributed to the suppression of pathogenic or spoilage bacteria, and, with cell autolysis, accelerated cheese ripening through the release of enzyme. There are species that convert non-carbohydrate compounds into aroma compounds such as diacetyl. The identified bacteria are *Lactiplantibacillus pentosus, Lactiplantibacillus plantarum*, *Lacticaseibacillus paracasei*, and *Lactiplantibacillus argentoratensis*.

Based on the results, we can confirm that some of the lactic acid bacteria isolated from fresh cow and goat milk cheese can be potentially applied as starter cultures in cheese production such as *Lacticaseibacillus paracasei* L13C, *Lactiplantibacillus pentosus* L10G, *Lactiplantibacillus plantarum* L7C, and *Lactiplantibacillus argentoratensis* L2C.

Keywords and phrases: lactic acid bacteria, cheese, technological aspects, salt tolerance, antibacterial activity

1. Introduction

Artisanal cheeses are part of the culinary culture of regions in the Eastern Carpathians of Transylvania, obtained from locally produced milk, using small-scale equipment and traditional processing methods (*Demeter et al.*, 2011). This fermented food has been part of that community diet since the beginning of their civilization. Particular quality of artisanal cheese is linked to natural resources of the region and to complex microbiota as native lactic acid bacteria (LAB) of milk (*Caldeira et al.*, 2024; *Rangel-Ortega et al.,* 2023). The primary role of starter cultures is to control the fermentation process. As a result of the conversion of lactose to lactic acid, the raw material transforms into fermented product (*Altieri et al.*, 2017; *Blaya et al.*, 2018; *Binda and Ouwehand*, 2019; *Ağagündüz et al.*, 2022; *Sharma et al.*, 2023). The autochthonous LAB are responsible for the specific and unique characteristics of artisanal cheese, including the technological, sensory, and safety traits. Due to their characteristics, they are involved in the maturation process, flavour (sensorial quality) and texture development. LAB play an important role in the secondary proteolysis of cheese, liberating low molecular weight peptides and free amino acids. These compounds serve as precursors in the formation of flavouring compounds contributing to more intense sensory properties. Bioactive peptides contribute to the safety of the product (*Guo and Liu* 2023; *Vallejo-Cordoba et al.*, 2023; *Abarquero et al.*, 2024; *Valdiviezo-Marcelo et al.*, 2023, *Hosken et al.*, 2023).

LAB are a group of bacteria with common metabolic and physiological characteristics. In general, these bacteria are Gram-positive, non-spore-forming bacteria, catalase and oxidase negative rods or cocci (*Deák* 2006; *von Wright and Axelsson*, 2019). The non-starter LAB are mesophilic LAB, mostly belonging to the genus *Lactobacillus*, but other species of *Pediococcus* or *Micrococcus* genera also appear (*Meng et al.*, 2018; *Grujović et al.*, 2022). They are involved in maturation through metabolic activity (proteolysis, amino acid catabolism, and lipolysis) and with enzymes released as a result of cell autolysis (*Carafa et al.*, 2016; *Randazzo et al.*, 2021; *Albayrak and Duran*, 2021; *Blaya, et al.*, 2018; *Chourasia et al.*, 2022).

The technological properties of LAB are acidifying capacity, growth at different temperatures, metabolic activity, production of different aroma and flavour compounds, or antimicrobial activity. The free amino acids are involved in flavour development. There is a correlation between cell autolysis and flavour development (*Altieri et al.*, 2017; *Abarquero et al.*, 2023).

In the process of selecting useful LAB strains for product development, the knowledge of species-specific metabolic and technological properties is an essential aspect. The limited number of strains from traditional cheese with excellent technological properties and the constant threat of bacteriophages encourages researchers to isolate new starter cultures. In addition, the new strains meet the

increasing and emerging consumer expectations and contribute to developing different product varieties with sensorial and nutritional complexity. The steps of starter selection involve the determination of technological and probiotic properties and their application at the laboratory and industrial level.

The indigenous microbiota of spontaneously fermented dairy products improves food safety by enhancing the technological and organoleptic functions of the product. These bacteria are competitive in the fermentation of traditional foods, preserving the sensory aspects of the product (*Domingos-Lopes et al.* 2017; *Wang et al.*, 2022; *Abarquero et al.*, 2022; *Abarquero et al.*, 2024).

The isolation and characterization of autochthonous bacteria from traditional products made by spontaneous fermentation contribute to the maintenance of microbiological diversity and to the production of new, higher-quality products relevant to a specific region (*Araújo-Rodrigue et al.*, 2021; *Ruvalcaba-Gómez et al.*, 2022; *Caldeira et al.*, 2024).

There is a lack of scientific information on the complex and diverse microbiota of artisanal cheeses. The selection of autochthonous starter cultures with desirable characteristics is the first tool to obtain a regionally specific cheese of lasting quality from milk from animals with a specific grassland habitat. The main objective of the present study was the selection and characterization of LAB isolated from locally obtained fresh cow and goat milk cheeses produced without commercially available starter cultures and traditional methods. During this study, the technological properties of the LAB, their resistance to antibiotics, and the detection of their antimicrobial effects were evaluated.

2. Materials and methods

2.1. Lactic acid bacteria isolation and identification

In the course of our work, more than 50 lactic acid bacteria isolates were isolated on de Man, Rogosa, and Sharpe (MRS Agar Fluka Analytical) agar from eight different artisanal fresh rennet-coagulated soft cheeses made from cow and goat milk without commercially available starter cultures. The cheese originates from the villages of the Csík Basin. This is one of the great tectonic basins of the Eastern Carpathians. From the samples, 10 g were smashed in a sterile mortar and homogenized with 90 ml physiological solution. From these samples, serial dilutions were made, and 0.1 ml from each mixture was spread on the prepared MRS agar medium surface. The inoculated media was incubated at 37 °C for 24 hrs in aerobic conditions. Fifty bacterial colonies with high numbers and characteristic colony morphology were isolated. Pure cultures were prepared.

Thirteen isolates were selected based on biochemical and phenotypic characteristics (salt tolerance, Gram staining, catalase test, acid and gas production) and were analysed for the most representative technological characteristics of LAB.

Total genomic DNA was extracted and purified according to the manufacturer's protocol (Bioneer AccuPrep® Genomic DNA Extraction Kit). Polymerase chain reaction for 16S rDNA amplification was performed as described by *György and Laslo* (2021).

2.2. Proteolytic activity

The proteolytic activity of LAB isolates was determined with two methods. The identified bacterial strains were inoculated on the surface of MRS supplemented with peptone 10 g/L and gelatine 30 g/L and incubated at 37 °C for 16–18 hrs followed by incubation at 4 °C for 5 hrs. The proteolytic activity of LAB was indicated by the turbidity that appeared around the colonies (*Landeta et al.*, 2013). The well diffusion method was used on modified skimmed milk agar medium (5 g/L casein, 2.5 g/L yeast extract, 1 g/L dextrose, 28 g/L skim milk powder, and 15 g/L agar). 50 µl of supernatant of bacterial isolates were inoculated in the hole in the modified skimmed milk containing agar medium. The incubation was performed at 37 °C for 48 hrs. The proteolytic activity was determined in accordance with the appeared clear zone surrounding each culture.

2.3. Acidifying activity

Acidifying activity was determined by monitoring pH change in 10 ml UHT milk inoculated with the tested LAB (1% v/v) and incubated at 37 °C. Over 48 hrs, the change in pH was measured (*Ribeiro et al.*, 2014).

2.4. Autolysis of LAB

The autolytic phenotype of identified LAB strains was evaluated in 50 mM/L pH 6.5 phosphate buffer. The buffer solution was inoculated with 2 g/100 ml LAB cultures. The optical density of the samples was measured at 600 nm for 72 hrs. The autolysis of lactic acid bacteria cells was expressed as percentage (%) of the initial change in optical density OD (OD 0/OD t*100, where OD 0 is the OD measured at the initial time point, and OD t is the OD measured at the time point under study) (*Mora et al.*, *2003*).

2.5. Diacetyl production

Diacetyl formation by the tested LAB was determined according to *Ribeiro et al.* (2014). An aliquot of 10 ml UHT milk was inoculated with the tested LAB (1%

v/v). The samples were incubated at 30 °C for 24 hrs. Subsequently, an amount of 0.5 ml of α-naphthol (1% w/v) and KOH solution (16% w/v) were added to one ml of each sample and incubated at 30 °C for 10 min. Diacetyl production was recorded by the forming of a red ring at the top of the tubes. The lighter red indicated medium capacity.

2.6. Antibacterial activity

The antibacterial activity of LAB cell-free supernatant was determined with the agar diffusion method on different pathogenic and spoilage indicator strains (from the microbiological laboratory of the University) as *Escherichia coli*, *Bacillus cereus*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Pseudomonas fluorescens*, and *Staphylococcus aureus*. The bacterial cultures were harvested for 24 hrs at 28 °C and 37 °C on nutrient agar. An amount of 0.1 ml bacterial suspension (with OD at 600 nm = 1) was spread on nutrient agar medium surface. 50 µl of the cell-free supernatant of LAB (14,000 rpm, 5 min) was dropped in the hole (d = 8 mm) cut with a sterile test-tube. The incubation was performed for 24 hrs at 28 °C / 37 °C. The antimicrobial effect of the LAB isolates was expressed in accordance with the diameter of the inhibition zone.

2.7. Antibiotic susceptibility

The determination of antimicrobial susceptibilities of LAB was performed according to the guideline reported in *EFSA* (2012). For the assessment of the susceptibility to nine antibiotics: penicillin, ampicillin, gentamicin, streptomycin, erythromycin, chloramphenicol, kanamycin, tetracycline, and clindamycin, two-fold serial dilutions were realized ranging from 0 up to 128 µg/ml in MRS broth *(Laslo et al.*, 2015).

2.8. Stability of lactic acid bacteria to freeze-drying

The stability of lactic acid bacteria to freeze-drying process was assessed using a modified method previously reported by *Carafa et al.* (2016). LAB from liquid culture was harvested by centrifugation (4,000 rpm for 20 min, 4 °C). The pellets were suspended in sterile freeze-drying medium and finally dried in a Cryodos 45 freeze dryer. For determination of the viability of freeze-dried LAB, an aliquot of 0.1 ml was suspended in a volume of 1 ml physiological solution. From the dilution series, 0.1 ml was spread on the surface of MRS agar medium. Incubation was performed at 37 °C for 24 hrs.

2.9. Statistical analysis

Analyses were performed in triplicate. The results of each determination were expressed as the mean ± standard deviation. Some results were analysed using principal component analysis, with the PAST software package (*https://past.en.lo4d.com/windows*), and some of the results were analysed with Tukey's test using IBM SPSS Statistics v22; $p < 0.05$ was considered statistically significant.

3. Results and discussions

Based on 16S rDNA, the selected 13 lactic acid bacterial strains from fresh cheese belong to the *Lactiplantibacillus* genus reclassified (*Zheng et al.*, 2020*)* (*Table 1*). The sequencing results showed that 38.46% of the total isolated bacteria were identified as *Lactiplantibacillus argentoratensis* (46.16%), as *Lactiplantibacillus pentosus* (7.69%), as *Lactiplantibacillus plantarum*, and as *Lacticaseibacillus paracasei* (7.69%).

Table 1. Molecular identification of the lactic acid bacteria isolates based on partial 16S rDNA analysis

Size in points	Isolates code	Bacterial source type of cheese	Most closely related organism	% Gene identity
1	L1C	Cow's milk cheese	*Lactiplantibacillus argentoratensis* ON527796.1	100%
2	L2C	Cow's milk cheese	*Lactiplantibacillus argentoratensis* ON527796.1	100%
3	L3C	Cow's milk cheese	*Lactiplantibacillus argentoratensis* ON527796.1	99.89%
4	L4C	Cow's milk cheese	*Lactiplantibacillus argentoratensis* ON527796.1	100%
5	L5C	Cow's milk cheese	*Lactiplantibacillus pentosus* ON387456.1	99.88%
6	L6C	Cow's milk cheese	*Lactiplantibacillus pentosus* MT229656.1	99.88%
7	L7C	Cow's milk cheese	*Lactiplantibacillus plantarum* CP052869.1	100%

Size in points	Isolates code	Bacterial source type of cheese	Most closely related organism	% Gene identity
8	L8G	Goat milk cheese	*Lactiplantibacillus argentoratensis* ON387453.1	99.89%
9	L9G	Goat milk cheese	*Lactiplantibacillus pentosus* ON495423.1	100%
10	L10G	Goat milk cheese	*Lactiplantibacillus pentosus* ON495423.1	99.89%
11	L11G	Goat milk cheese	*Lactiplantibacillus pentosus* ON495423.1	100%
12	L12C	Cows' milk cheese	*Lactiplantibacillus pentosus* ON495423.1	99.88%
13	L13C	Cows' milk cheese	*Lacticaseibacillus paracasei* ON387664.1	100%

Traditional foods obtained by spontaneous fermentation are a promising source of new, competitive starter cultures. The quality, flavour of artisanal cheese highly correlates with bacterial diversity (*Zhang et al.*, 2021; *Grujović et al.*, 2022; *Guo and Liu* 2023). In the different types of cheese, *Lactiplantibacillus plantarum* was the most frequently occurring non-starter LAB*. L. plantarum subsp. plantarum* and *L. plantarum subsp. argentoratensis* are known for their health benefits as probiotics, as they are safe and thus urged to be used in dietary supplements or dairy products (*Choi et al.*, 2021; *Yilmaz et al.*, 2022; *Guo and Liu* 2023).

The studied strains cultured in media with protein supplementation showed different proteolytic activities. These strains have different proteolytic capacities depending on the used proteins. 38% of the studied bacterial strains showed the most favourable result. *Lactiplantibacillus argentoratensis* L2C*, Lactiplantibacillus pentosus* L6C*, Lactiplantibacillus pentosus* L9G, *Lactiplantibacillus pentosus* L10G, and *Lactiplantibacillus pentosus* L11G could hydrolase the most of the proteins, and the halozone was the most interpretable in these cases.

During ripening, proteolysis is a complex biochemical process that determines the texture and softness of cheese (*Ribeiro et al.*, 2014; *Pagthinathan and Nafees*, 2015). LAB proteinases and peptidases produce peptides and amino acids during ripening (*Ardö et al.*, 2017). These compounds serve as precursors for flavour development. Autochthonous LAB has been shown to contribute to higher proteolysis in traditional cheese products (*de Aguiar e Câmara et al.*,

2022). *Lb. plantarum* strains in yogurt showed the highest proteolytic activity and contributed to developing texture and volatile flavour (*Yilmaz et al.*, 2022).

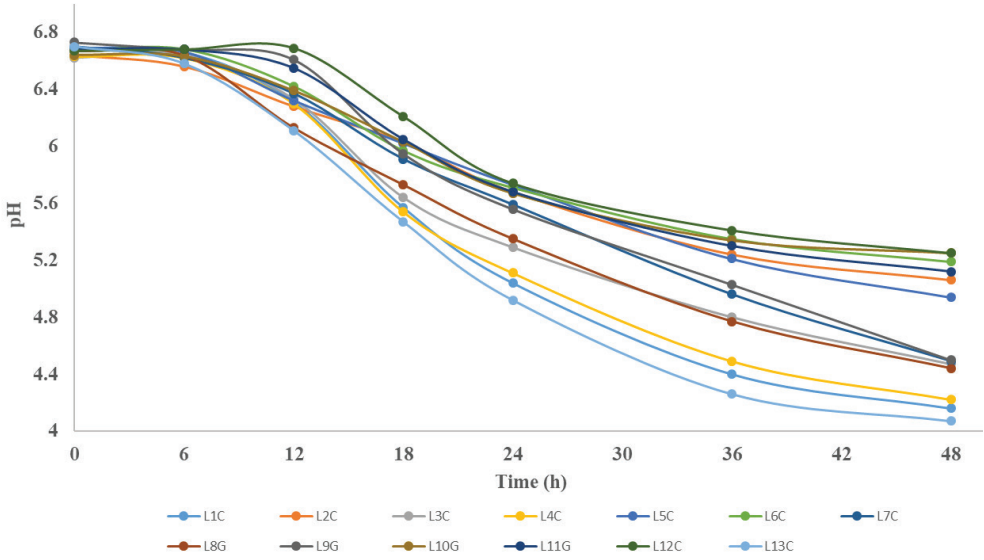

Figure 1. Acidifying capacity of the studied bacterial strains

The pH values of the inoculated UHT milk are shown in *Figure 1*. The majority of the bacterial strains were the slowest acid producers. In this case, it took 36 or 48 hours for the pH to decrease below 5. *Lacticaseibacillus paracasei* L13C reduced the pH of the UHT milk after 24 hrs. The pH values after 18 hrs were 5.57 in the case of *Lactiplantibacillus argentoratensis* L1C, 5.64 in the case of *Lactiplantibacillus argentoratensis* L3C, 5.54 in the case of *Lactiplantibacillus argentoratensis* L4C, and the *Lactiplantibacillus argentoratensis* L8G reduced the pH to 5.73. After 48 hours, the pH values were approximately 4 for three strains: 4 in the case of *Lacticaseibacillus paracasei* L13C, 4.16 in the case of *Lactiplantibacillus argentoratensis* L1C, and for *Lactiplantibacillus argentoratensis* L4C it was 4.22. The mentioned bacterial strains have an excellent acidifying capacity as starter cultures for cheese. This is due to their ability to reduce the pH by 0.05–0.2 within the first 120 minutes, which is favourable for different types of cheese.

Acid production is one of the fundamental properties of starter cultures. In some cheeses, it can contribute to sensory defects. For coagulation, denaturation, firmness of the curd/cheese, and control of undesirable microbes, it is essential to lower the pH as quickly as possible. Generally, *Lactiplantibacillus* species are moderate acidifiers, with some of them being slow (*Ribeiro et al.*, 2013; *Todorov et al.*, 2017; *Meng et al.*, 2018).

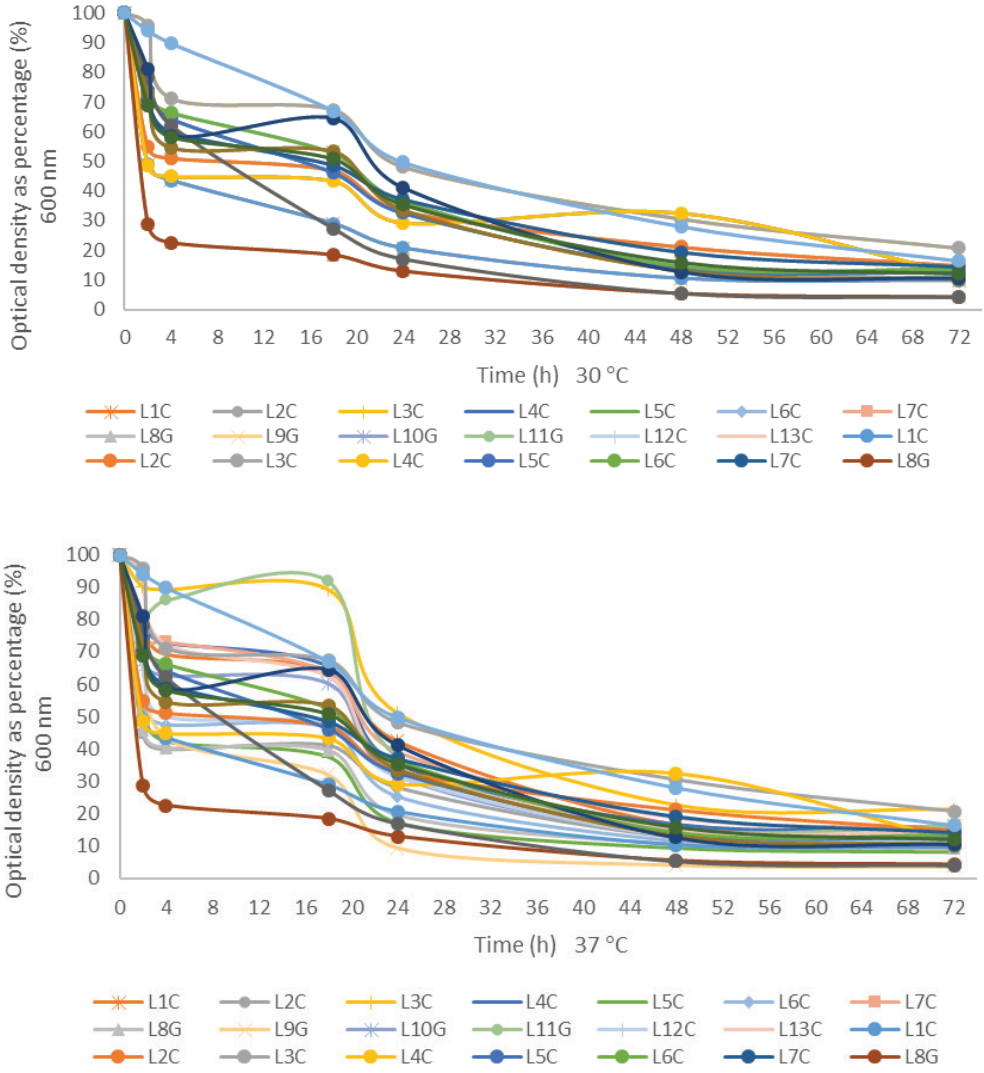

Figure 2. Autolysis of the LAB cells cultured at 30 °C (*A*) and at 37 °C (*B*)

Another technological aspect of LAB is cell autolysis. The importance of this lies in the release of intracellular enzymes. Autolyzed cells have been shown to accelerate peptidase activity and reduce the bitterness of cheese (*Cogan and Beresford, 2002*; *Cheng et al., 2022*). Cell autolysis accelerates cheese ripening through the release of enzymes. Generally, there is no substantial difference in the autolytic ability of the tested strains at 30 °C and 37 °C. For *Lactiplantibacillus argentoratensis* L8G, autolysis rates of at least 22.58% were observed after four hours at 30 °C and 45.65% at 37 °C. The highest percentage decrease in OD at

30 °C was found for *Lactiplantibacillus argentoratensis* L8G (12.962 g / 100 ml) and the lowest for *Lacticaseibacillus paracasei* L13C (49.683%).

After 48 hrs, the lowest change in relative OD was 32.5% for *Lactiplantibacillus argentoratensis* L4C, while autolysis was 5.278% for *Lactiplantibacillus pentosus* L9G.

After 72 hrs, the autolysis of *Lactiplantibacillus argentoratensis* L3C was 20.649% and 4 g/100ml for *Lactiplantibacillus pentosus* L9G (*Figure 2*).

For one strain, *Lactiplantibacillus argentoratensis* L2C, at least 39.85% autolysis was detected at 37 °C after 4 hrs. The percentage decrease in the OD at 37 °C after 24 hrs for *Lactiplantibacillus argentoratensis* L3C was 50.943% and 9.444 g/100ml in the case of *Lactiplantibacillus pentosus* L9G. After 48 hrs, the highest decrease in OD was observed for *Lactiplantibacillus argentoratensis* L3C (22.594 g / 100 ml). The lowest reduction in OD was observed for *Lactiplantibacillus pentosus* L9G (4.086%).

After 72 hours, the autolysis ability, expressed as the change in OD, varied from 21.259% for *Lactiplantibacillus pentosus* L9G to 3.640% for *Lactiplantibacillus argentoratensis* L3C.

Regarding the growth ability at different temperatures, it can be noted that the bacterial strains grew slightly at 4 and 45 °C. At 15 °C, the OD values increased three times and four to five times at 37 °C compared to the initial OD. The highest growth was observed at 30 °C (data not shown). The results indicate that the tested cultures are mesophilic bacteria due to their strong growth at 30 °C.

Lactic acid bacteria produce different aroma compounds that contribute to the formation of the flavour and aroma of the product. The diacetyl concentration was the highest in the case of *Lacticaseibacillus paracasei* L13C and *Lactiplantibacillus plantarum* L7C. For the other bacterial strains, diacetyl production was moderate, as the positive result appeared after a few hours. LAB contribute to the development of specific organoleptic characteristics of certain cheeses by producing this aroma compound. The aroma production in Caciocavallo cheese by *Lactocaseibacillus plantarum* and *Lactocaseibacillus paracasei* is attributed to the low activity of aminopeptidase type N and cystathionine lyase (*Yavuz et al., 2021*).

These microorganisms are capable of converting non-carbohydrate compounds into aroma compounds, such as diacetyl and acetoin, through their metabolism (*Ruiz Rodríguez et al., 2017*). *Lazzi et al.* (2016) found that the intracellular enzymes released during the autolysis of LAB were mainly involved in the cheese aroma formation in some specific cheese varieties. Estherolytic and proteolytic activities enhance the flavour production of lactic acid bacteria (*Albayrak and Duran*, 2021).

The antibacterial activity of the bacterial strains was evaluated using the agar diffusion method against six Gram-positive and Gram-negative bacteria. The effects of the studied LAB supernatant are shown in *Table 2*.

When selecting starter cultures, one consideration is the antimicrobial activity of the bacterial strains. With this potential, LAB contribute to suppressing pathogenic or spoilage bacteria.

Table 2. Antimicrobial effect of lactic acid bacteria on Gram-positive and Gram-negative bacteria

LAB strain	*Bacillus cereus*	*Bacillus subtilis*	*Escherichia coli*	*Pseudomonas aeruginosa*	*Pseudomonas fluorescens*	*Staphylococcus aureus*
	Inhibition zone diameter ± SD (mm)					
Lactiplantibacillus argentoratensis L1C	12.5 ± 0.5^{d}	16 ± 0^{cd}	10.5 ± 0.5^{cd}	10 ± 0^{a}	20.5 ± 0^{f}	11.5 ± 0.5^{bcd}
Lactiplantibacillus argentoratensis L2C	10.5 ± 0.5^{c}	17 ± 0^{d}	11 ± 0^{cde}	22.5 ± 2.5^{f}	20.5 ± 0.5^{f}	11.5 ± 0.5^{bcd}
Lactiplantibacillus argentoratensis L3C	7.5 ± 0.5 ^{ab}	15 ± 0^{c}	0 ± 0^{a}	18 ± 0^{cd}	19 ± 1^{def}	10.5 ± 0.5^{abc}
Lactiplantibacillus argentoratensis L4C	6.5 ± 0.5 ^{a}	15.5 ± 0.5^{c}	10 ± 1 ^{c}	16.5 ± 0.5 ^{bc}	17.5 ± 2.5^{bcd}	11.5 ± 0.5^{bcd}
Lactiplantibacillus pentosus L5C	7.5 ± 0.5^{ab}	15 ± 0^{c}	12.5 ± 1.5^{ef}	21 ± 1^{def}	16.5 ± 0.5^{bc}	11.5 ± 1.5^{bcd}
Lactiplantibacillus pentosus L6C	12 ± 0 ^{cd}	11 ± 0^{b}	10.5 ± 0.5^{cd}	10 ± 0^{a}	18 ± 1^{cde}	8.5 ± 0.5^{a}
Lactiplantibacillus plantarum L7C	7.75 ± 0.75^{ab}	15.5 ± 0.5^{c}	10 ± 0^{c}	17.5 ± 0.5^{c}	15.5 ± 0.5^{ab}	11.5 ± 0.5^{bcd}
Lactiplantibacillus argentoratensis L8G	8.25 ± 0.25^{ab}	12 ± 0.5 ^{b}	12 ± 1^{def}	18 ± 0^{cd}	14 ± 0^{a}	11 ± 1^{bcd}
Lactiplantibacillus pentosus L9G	6.5 ± 0.5^{a}	16 ± 0^{cd}	7 ± 0^{b}	15.5 ± 0.5^{bc}	20 ± 0^{ef}	10 ± 0^{ab}
Lactiplantibacillus pentosus L10G	8.5 ± 0.5^{b}	16 ± 0^{cd}	13.5 ± 0.5 ^{f}	21.5 ± 0.5^{ef}	18 ± 0^{cde}	13 ± 1^{d}
Lactiplantibacillus pentosus L11G	11 ± 1^{cd}	16 ± 0^{cd}	10.5 ± 0.5^{cd}	24 ± 0^{f}	25 ± 0^{g}	12 ± 1^{bcd}
Lactiplantibacillus pentosus L12C	12 ± 1^{cd}	13 ± 0.5^{b}	12.5 ± 0.5^{ef}	13.5 ± 1.5^{b}	20 ± 0^{ef}	12.5 ± 0.5^{cd}
Lacticaseibacillus paracasei L13C	10.5 ± 0.5^{c}	0 ± 0^{a}	10.5 ± 0.5^{cd}	18.5 ± 1.5^{cde}	20.5 ± 0^{f}	8.5 ± 0.5^{a}

Note: Means ± standard deviation with different letters are significantly different by Tukey's test ($p < 0.05$).

The antimicrobial activity is exerted by various mechanisms due to their metabolites including dissociated or undissociated organic acids, bacteriocins, etc. The growth-inhibitory activity is due to the termination of the transmembrane proton motive force (*Laslo et al.*, 2020). Based on the inhibition zone, a greater inhibitory effect against *Bacillus cereus* was observed in the case of the cell-free supernatant of *Lactiplantibacillus argentoratensis* L1C and the supernatant of two strains of *Lactiplantibacillus pentosus* L11G and *Lactiplantibacillus pentosus* L12C. *Bacillus subtilis* was strongly inhibited by the supernatant of *Lactiplantibacillus argentoratensis* L2C. The cell-free supernatant of four strains inhibited *Bacillus subtilis* at the same level. *Lactiplantibacillus pentosus* L10G exhibited a strong inhibitory effect against *E. coli*. Weak antibacterial effect was detected in the cases of *Lactiplantibacillus pentosus* L12C and *Lactiplantibacillus pentosus* L5C. The strongest antibacterial effect was observed against *Pseudomonas aeruginosa. Pseudomonas fluorescens and Ps. aeruginosa* were the most inhibited by the cell-free supernatant of *Lactiplantibacillus pentosus* L11G. The following bacteria were also found to have a strong antibacterial effect: *Lactiplantibacillus argentoratensis* L2C and *Lactiplantibacillus pentosus* L10G. The largest inhibition zone was observed in the case of *Lactiplantibacillus pentosus* L11G against *Ps. fluorescens*.

Intense antibacterial activity was also observed for *Lacticaseibacillus paracasei* L13C, *Lactiplantibacillus argentoratensis* L1C, and *Lactiplantibacillus argentoratensis* L2C. *Staphylococcus aureus* was weakly inhibited by the tested lactic acid bacteria. The highest level of inhibition was observed in *Lactiplantibacillus pentosus* L10G, with a slightly lower level observed in *Lactiplantibacillus pentosus* L12C. For this indicator bacterium, the antibacterial effect was weaker compared to the others. Similar results regarding the antibacterial efficacy of autochthonous lactic acid bacteria have also been described in the literature (*Araújo-Rodrigues et al.*, 2021; *Glieca et al.*, 2024).

LAB serve as biocontrol agents against potentially pathogenic bacteria. *L. plantarum* strains have been shown to have antimicrobial activity against Gram-negative and Gram-positive bacteria and moulds that can contaminate food and cause human diseases (*Russo et al.*, 2017; *Arena et al.*, 2016). Among our bacterial strains, three *Lactiplantibacillus pentosus* strains based on inhibition zone showed more substantial antibacterial effect against *E. coli* and mild effect against *Staphylococcus aureus* and *Bacillus cereus* compared to the results of 48 hrs (*Motahari et al.*, 2017).

Survival and stability to freeze-drying were performed for four bacterial species from the studied strains: *Lactiplantibacillus pentosus* L9G, *Lacticaseibacillus paracasei* L13C, *Lactiplantibacillus pentosus* L10G, and *Lactiplantibacillus argentoratensis* L2C.

Freeze-drying is a widely used method for the formulation of starter cultures due to its advantages in maintaining biological activity and convenient storage

at room temperature (*Chen et al.*, 2021). After freeze-drying, the cell number was $2.1 \cdot 10^{12}$ CFU/g in the case of *Lactiplantibacillus pentosus* L9G and $1.3 \cdot 10^{12}$ CFU/g in the case of *Lacticaseibacillus paracasei* L13C. In the case of *Lactiplantibacillus pentosus* L10G, $1.35 \cdot 10^{12}$ CFU/g was detected and in the case of *Lactiplantibacillus argentoratensis* L2C $6 \cdot 10^{12}$ CFU/g after freeze-drying. The results show that the viability of the four tested species was not affected during freeze-drying.

Multivariate analysis of technological LAB characteristics was performed using principal component analysis (PCA). PCA results showed that the first two components accounted for 89.01% of the total variance. The first component, which accounted for 76.391% of the total variance, had the highest eigenvalue of 2.241, and the second component accounted for 12.62 % of the total variance with an eigenvalue of 0.370.

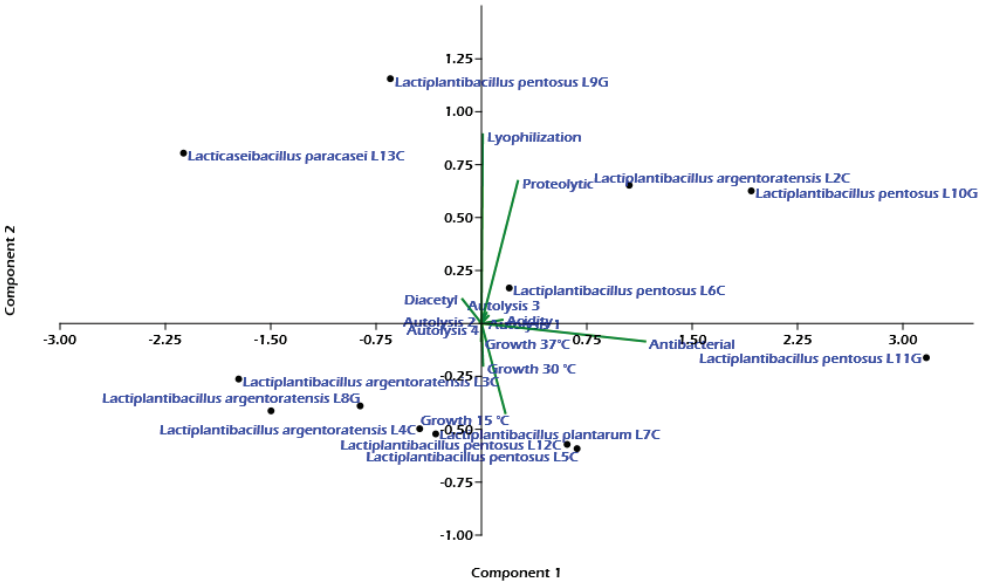

Figure 3. Principal component analysis biplot based on the technological properties of identified lactic acid bacteria

LAB whose characteristics highly correlate cluster together. On the right side in quadrant I, there are three LAB *Lactiplantibacillus pentosus* L10G, *Lactiplantibacillus argentoratensis* L2C, and *Lactiplantibacillus pentosus* L6C, and in the right quadrant IV there are *Lactiplantibacillus pentosus* L5C, *Lactiplantibacillus pentosus* L12C, and *Lactiplantibacillus pentosus* 11G. In left side in quadrant II , there are *Lactiplantibacillus pentosus* L13C and *Lactiplantibacillus pentosus* L9G, the rest of the characterized bacteria being grouped in the left quadrant III. With regard to the safety of the starter culture, the antibiotic resistance

is important. LAB can have multiple antibiotic resistance, which phenomenon occurs naturally or is genetically acquired (*Guo et al.*, 2017). Antibiotic resistance is species- and strain-dependent. Antibiotic susceptibility was highly dependent on the isolated strain. Based on the phenotypic antibiotic resistance determination, it can be concluded that among the most promising lactic acid bacteria, *Lacticaseibacillus paracasei* L13C was susceptible to penicillin, ampicillin, and clindamycin. The minimum inhibitory concentration (MIC) value was 1 μg/mL in three cases. For the other six antibiotics, the MIC was 128 or greater than 128 μg/ml. *Lactiplantibacillus pentosus* L10G was susceptible to penicillin – the MIC value was 1 μg/mL; for ampicillin: MIC = 1 μg/mL, clindamycin: MIC = 2 μg/mL, tetracycline: MIC = 32 μg/mL, and kanamycin: MIC = 64 μg/mL. *Lactiplantibacillus argentoratensis* L2C was susceptible to penicillin: MIC = 8 μg/mL, ampicillin: MIC = 1 μg/mL, clindamycin: MIC = 2 μg/mL, and tetracycline: MIC = 32 μg/mL. The MIC for streptomycin, kanamycin, erythromycin, chloramphenicol, and gentamicin was greater than 128 μg/ml. The identified bacterial strains have MIC > 128 μg/mL for streptomycin, erytromicin, chloramphenicol, gentamicin, and tetracycline. Our results are similar, with minor differences, for the bacteria described by *Radulović et al.* (2010) and *Zarzecka et al.* (2022).

It has been shown that the genes for resistance to erythromycin, tetracycline, and chloramphenicol are often found on mobile genetic elements in lactic acid bacteria. The possibility of gene transfer by lactic acid bacteria is a concern, and antibiotic resistance of starter or adjunct cultures is undesirable (*Dušková et al.*, 2020; *Zarzecka et al.*, 2022). Further research is needed to determine the presence of antibiotic resistance genes.

Table 3. Minimum inhibitory concentrations (MIC μg/mL) of the LAB strains tested for nine different antibiotics

LAB	**Antibiotics μg/mL**								
	Penicillin	**Streptomycin**	**Ampicillin**	**Kanamycin**	**Clindamycin**	**Erythromycin**	**Chloramphenicol**	**Gentamicin**	**Tetracycline**
Lacticaseibacillus paracasei L13C	1	128	1	128	1	> 128	> 128	> 128	> 128
Lactiplantibacillus pentosus L10G	1	> 128	1	64	2	> 128	> 128	> 128	32
Lactiplantibacillus argentoratensis L2C	8	> 128	1	> 128	2	> 128	> 128	> 128	32

4. Conclusions

Naturally fermented traditional cheeses are promising sources for the selection of novel starter cultures. The autochthonous microflora of cow's and goat's milk cheeses produced in our region without commercial starter cultures includes the *Lactiplantibacillus* and *Lacticaseibacillus* species. The results showed that the majority of the strains exhibited proteolytic, antibacterial, and autolysis activity. The lactic acid bacteria tested were moderate acid producers, grew well at different temperatures, and showed stability to the freeze-drying process.

Based on this study, *Lactiplantibacillus pentosus* L10G, *Lactiplantibacillus plantarum* L7C, *Lacticaseibacillus paracasei* L13C, and *Lactiplantibacillus argentoratensis* L2C with good technological properties were found to be promising candidates for the development of cheese or fermented dairy products after further safety assessment as antibiotic-resistant genes.

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Formulation and impact of soy protein isolate on white oyster mushroom (*Pleurotus ostreatus*) sausage: Palatability evaluation, nutritional and economic value

A. Masnar[1]
e-mail: asriadi.masnar@polimedia.ac.id

R. T. Haryati[2]
e-mail: ratih.haryati@dinus.id

M. A. Akbar[1]
e-mail: m.akbar@polimedia.ac.id

[1] Culinary Department, State Polytechnic of Creative Media, Jakarta, Indonesia
[2] Hospitality Management Dian Nuswantoro University, Semarang, Indonesia

Abstract. This study investigated the utilization of white oyster mushrooms (*Pleurotus ostreatus*) and soy protein isolate in developing vegetarian sausages. Two formulations were evaluated: a control (100% mushrooms) and an experimental (80% mushrooms, 20% soy protein isolate). Sensory assessment using a 5-point hedonic scale with 25 trained panellists revealed no significant differences ($p > 0.05$) in aroma, taste, texture, or colour between formulations. Chemical analysis indicated that the experimental sample showed a significant increase in protein content from 0.42% to 2.24%, though this still falls short of the Indonesian National Standard (SNI 3820:2015) minimum requirement of 8% for sausages. Moisture, fat, and ash content were comparable between formulations and within SNI limits. Carbohydrate content was slightly lower in the experimental sausages but presumably exceeded the SNI minimum. Microbiological testing demonstrated that both formulations met safety standards, with total plate count, coliform count, and *Salmonella* levels well below the limits set by SNI 3820:2015. Cost estimation revealed that the vegetarian sausages had a lower selling price compared to traditional meat sausages, offering an economically viable alternative. This research highlights the potential of mushrooms and plant proteins in developing nutritious, safe, and cost-effective meat alternatives though further formulation improvements are needed to meet protein content standards for sausage products.

Keywords and phrases: meat analogue, alternative protein sources, sensory evaluation, hedonic test

1. Introduction

Sausage is a popular processed food, usually made from meat or a combination of several types of meat through a process of mashing and mixing with herbs or spices. The main ingredients of sausages usually come from beef, pork, chicken, fish, and rabbit (*Leroy et al.*, 2006). From year to year, the demand for ready-to-cook food is getting higher. This is validated by survey data obtained in the framework of the National Economy Survey in 2018, suggesting that the consumption of processed meat, including sausages, in some regions of Indonesia takes place almost on a monthly basis (*Rasyda & Santosa*, 2023). The demand for meat also increased along with the high demand for sausages in the market. The Chairman of the Indonesian Meat Processing Industry Association and National Meat Processor Association stated that the income of the meat processing industry per day had a demand for chicken meat of 75 metric tons and an income range of one trillion Indonesian Rupiah (IDR) per year (*Hugo & Hugo*, 2015).

The other side of the popularity of consuming processed meat products, such as sausages, is that people who consume them excessively contribute to increasing the mortality rate in favour of cardiovascular diseases and cancer. Faced with such a reality, people want to live healthier but do not want to change their consumption patterns such as reducing processed meat products to consume healthier foods. Vegetarianism is currently one of the healthy lifestyle choices adopted in various countries, including Indonesia. It can be defined as avoiding foods that contain meat or animal products. Therefore, avoiding and reducing animal products is part of a healthy lifestyle (*Nezlek & Forestell*, 2020; *Rosenfeld*, 2018). However, there are also contrary opinions stating that a balanced, moderate consumption of processed meats can be part of a healthy diet. Critics argue that eliminating food groups is unnecessary and can lead to nutritional deficiencies if not properly planned. They advocate for reasonable portion control of meat products rather than strict avoidance. Nevertheless, developing appetizing plant-based meat alternatives provides options for those looking to reduce processed meat intake without drastically altering their dietary patterns.

The obstacles experienced in following a vegetarian diet are the temptation of non-vegetarian food and the influence of a non-vegetarian environment. Vegetarians feel confused to find a substitute for the protein found in meat, which is their biggest problem (*Permana & Dewanto*, 2021). It can be concluded that people tend to want to have a healthier lifestyle while still wanting to eat foods whose consumption they should reduce, namely meat products or their preparations. One of the vegetarian food ingredients that can be used as a substitute for meat products in animals is oyster mushrooms (*Giawa*, 2023).

Oyster mushrooms have beneficial effects because they do not contain cholesterol. Oyster mushrooms can be a food alternative to meat raw materials (*Gizatova et*

al., 2020). To increase the protein content in oyster mushrooms, other ingredients are needed, one of which is soy protein isolate flour. Soy protein isolate is a food product resulting from the separation process of oil, water, starch, and carbohydrate components of soybeans with the aim of achieving protein levels (*Munasir & Sekartini*, 2020). Soy protein isolate is one of the numerous ingredients that can be utilized as a binder in the production of processed meat products due to its ability to bind both water and oil. It can stabilize emulsions and help to maintain the structure or shape of processed meat products (*Gao et al.*, 2015).

Sausage is one of the processed meat products the demand for which is increasing year by year in the community. On the contrary, excessive consumption of processed meat products such as sausages is associated with increased risk of mortality, and there is a limited choice of alternative protein sources for meat products. The authors aimed to formulate a white oyster mushroom vegetarian sausage using soy protein isolate as alternative vegetarian sausage products.

2. Materials and methods

Materials

White oyster mushroom, isolate protein soybeans (Para Agribusiness), salt (Segitiga Biru,® Indonesia), sugar (Gulaku,® Indonesia), ground white pepper (Abrofood,® Indonesia), tapioca (Rosebrand,® Indonesia), crystal ice (Air Beku,® Indonesia), palm cooking oil (Bimoli,® Indonesia), garlic powder (Koepoe Koepoe,® Indonesia), sodium tripolyphosphate (Aditya Birla,® Indo Food Chem, Indonesia), polyamide sausage casings (skin) (Devro,® Czech Republic), and butchers cotton twine (Librett Durables,® USA) were purchased through online trading. The product was prepared in the kitchen laboratory at Polimedia Campus, Jakarta.

Methods

This research uses quantitative methods with random sampling techniques. Normality was assessed using the Shapiro–Wilk test. The validity of the measurement instrument used was assessed by examining the significance level of each item. An item was considered valid if its significance level was below 0.05. The reliability of the instrument was evaluated using Cronbach's alpha (α), with a value greater than 0.6 indicating acceptable reliability.

The vegetarian sausage products were prepared (*Table 1*) using two different formulations, A and B. Formula A1/A2 consists of 100% white oyster mushrooms and 0% soy protein isolate. Formula B1/B2 is composed of 80% white oyster mushrooms and 20% soy protein isolate. The numbers in parentheses next to

each formula variation (745, 467, 905, and 275) are random numbers associated with the formulas.

Table 1. Composition of the sausage formula

No	Ingredient name (English)	Unit	Control	Experiment
1.	White oyster mushroom	g	100	80
2.	Soy protein isolate (SPI)	g	0	20
3.	Tapioca flour	g	15	15
4.	Sodium tripolyphosphate (STPP)	g	0.5	0.5
5.	Garlic powder	g	0.2	0.2
6.	Salt	g	3	3
7.	Pepper	g	0.2	0.2
8.	Sugar	g	1	1
9.	Cooking oil	ml	20	20
10.	Distilled water	ml	25	25

Sensory evaluation

The outcomes of this experiment were evaluated using a hedonic test to assess the sensory attributes and consumer acceptance. This evaluation included the level of liking with a numerical representation that incorporated aspects of product evaluation from the human sensory indicators of aroma, taste, texture, and colour. The test was conducted in a provisional laboratory at Jakarta State Polytechnic of Creative Media Campus, located in Srengseng Sawah, South Jakarta. A total of 25 trained panellists, consisting of students from the Culinary Arts Study Programme, participated in the study. These panellists had previously undergone a triangle test, which is a discriminatory test in sensory evaluation. In the triangle test, a set of three samples is presented to the panellists, who must identify the odd sample without using a reference, to assess their sensory discrimination abilities.

Data measurement and analysis

The hedonic test was conducted according to the favourability level and scored accordingly: dislike extremely (1), dislike (2), quite like (3), like (4), like extremely (5) (*Mello et al.*, 2019). The mean was obtained using the formula:

$$\overline{x} = \frac{\sum f(x)}{n} \tag{1}$$

Notation:
$\overline{x}$ = mean (average),
$\Sigma f(x)$ = number of frequencies multiplied by the value,
n = number of panellists.

The average score criteria, as a method by *Lim* (2011), was done by calculating the interval value, that is, the highest score minus the lowest score, and the result was divided by the number of types of assessment criteria. The result is 0.8 – hence, the intervals are 1–1.8 (dislike extremely), 1.9–2.6 (dislike), 2.7–3.4 (moderately like), 3.5–4.2 (like), and 4.3–5 (like extremely).

Product development and work steps

The experimental process consisted of two formulations, with two repetitions. The production process was based on the following flowchart (*Figure 1*).

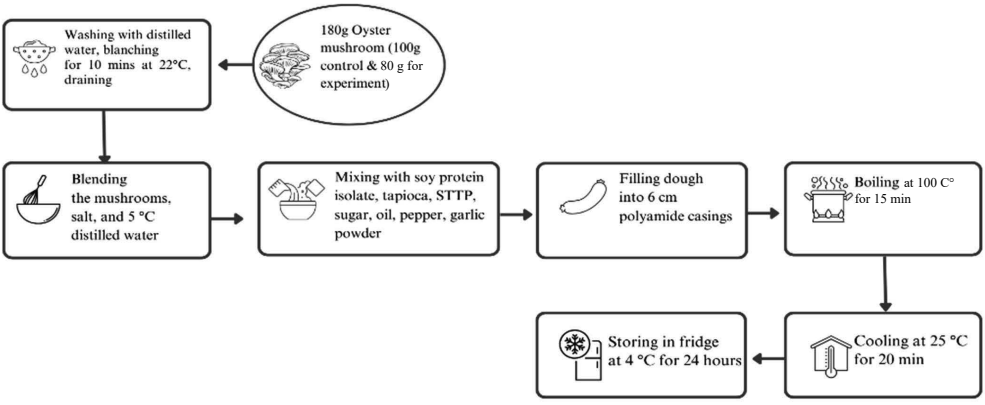

Figure 1. Flowchart of white oyster mushroom sausage formulation

Data processing and analysis

The hedonic data were collected and analysed with SPSS (Statistical Package for the Social Sciences) version 25. The data were not normally distributed; hence, the Mann–Whitney U test was performed.

Number of trial units

Experiments were performed twice for this formulation. The number of experimental units (*n*) was expressed as a product between the number of repetitions (*r*) and the number of treatments (*t*).

Chemical analysis and microbiological evaluation

Moisture content determination (SNI 2354.2:2015)

2 g of sausage sample was weighed into a pre-weighed aluminium dish. The sample was dried in an oven at 105 °C for 3 hours or until constant weight was achieved. The dish was cooled in a desiccator and reweighed. Moisture content was expressed as mass percent.

Ash content determination (SNI 2354.1:2010)

2 g of sausage sample was weighed into a pre-weighed porcelain crucible. The sample was incinerated in a muffle furnace at 550 °C for 5 hours or until white ash was obtained. The crucible was cooled in a desiccator and reweighed. Ash content was expressed as mass percent.

Protein content determination (Kjeldahl method)

1 g of sausage sample was weighed into a Kjeldahl flask. 15 ml of concentrated sulphuric acid and a catalyst tablet ($K_2SO_4 + CuSO_4$) were added. The mixture was digested at 420 °C for 1 hour or until the solution became clear. After cooling, the sample was distilled with 40% NaOH solution. The distillate was collected in 4% boric acid solution with indicator and titrated with standardized 0.1 N HCl. Nitrogen content was calculated and multiplied by 6.25 to obtain protein content.

Fat content determination (Soxhlet extraction method)

5 g of dried sausage sample was weighed into an extraction thimble. The sample was extracted with petroleum ether in a Soxhlet apparatus for 6 hours. The extract was dried and weighed. Fat content was expressed as mass percent.

Total plate count (TPC) (SNI 01-2332.3:2006)

Serial dilutions of the sausage sample were prepared in sterile peptone water. 1 ml of appropriate dilutions was inoculated onto Plate Count Agar using the pour

plate method. The plates were incubated at 35 °C for 48 hours. Colonies were counted and reported as CFU/g.

Escherichia coli detection (SNI 01-2332.1:2006)

1 ml of appropriate sausage sample dilutions was inoculated into Lauryl Sulphate Tryptose (LST) broth. The broth was incubated at 35 °C for 24-48 hours. A loopful from positive LST tubes was transferred to EC broth and incubated at 44.5 °C for 24 hours. Positive EC tubes were streaked onto Eosin Methylene Blue (EMB) agar. Typical colonies were confirmed with biochemical tests. Results were reported as MPN/g.

Salmonella detection (SNI 01-2332.2:2006)

25 g of sausage sample was pre-enriched in 225 ml Buffered Peptone Water at 35 °C for 24 hours. 0.1 ml was transferred to 10 ml Rappaport-Vassiliadis (RV) broth and 1 ml to 10 ml Tetrathionate (TT) broth. RV was incubated at 42 °C and TT at 35 °C for 24 hours. The broths were streaked onto Xylose Lysine Deoxycholate (XLD) and Bismuth Sulphite (BS) agars and incubated at 35 °C for 24 hours. Typical colonies were confirmed with biochemical and serological tests. Results were reported as presence or absence in 25 g.

3. Results and discussions

The development of the vegetarian sausage focused on creating a plant-based alternative that mimics the sensory and nutritional qualities of traditional meat sausages. Two formulations were developed: a control formulation using 100% white oyster mushrooms (*Pleurotus ostreatus*) and an experimental formulation comprising 80% white oyster mushrooms and 20% soy protein isolate. White oyster mushrooms were chosen as the primary ingredient due to their meat-like texture and umami flavour profile (*Giawa*, 2023), while soy protein isolate was selected to enhance the protein content and improve the binding properties of the mixture (*Gao et al.*, 2015). Additional ingredients common to sausage production were incorporated, including tapioca starch (5%) as a binder and texture enhancer (*Mazumder et al.*, 2023), salt (1.5%) for flavour enhancement and preservation, sugar (0.5%) to balance flavours, white pepper (0.3%) and garlic powder (0.2%) for seasoning, and sodium tripolyphosphate (0.3%) to improve water retention and texture (*Hugo & Hugo*, 2015).

The sausage production process involved several steps: preparation of ingredients, mixing, emulsification, stuffing into polyamide casings (19 mm diameter), linking to

6 cm lengths, cooking in water at 80 °C for 30 minutes until an internal temperature of 72 °C was reached, cooling in an ice bath, and, finally, vacuum-packing and refrigerating at 4 °C. The final products had a length of 6 cm, a diameter of 19 mm, and weighed approximately 15 g per link. Visually, both formulations resembled traditional sausages, with the experimental formulation showing a slightly firmer texture due to the soy protein isolation. The colour of both formulations was light beige before cooking, turning to golden brown after frying, similar to the colour change observed by *Kang et al.* (2022) in their study on plant-based sausages.

During development, several challenges were addressed. Initial formulations were too moist, leading to a soft texture, which was corrected by adjusting the ratio of mushrooms to dry ingredients and optimizing the cooking process. The control formulation initially had poor binding properties, which was improved by increasing the tapioca starch content and fine-tuning the emulsification process. Early versions lacked the umami flavour associated with meat sausages, addressed by adjusting the seasoning profile and incorporating a small amount of yeast extract (0.1%) to enhance savoury notes. The experimental formulation initially had a slightly grainy texture due to the soy protein isolate, which was resolved by hydrating the latter before mixing. These optimizations resulted in two formulations that closely resembled traditional meat sausages in appearance and texture, while offering the nutritional benefits of plant-based ingredients.

The Shapiro–Wilk test results indicate a non-normal distribution ($p < 0.05$) for the sensory attributes of aroma, taste, texture, and colour in both the control and experimental groups. Specifically, p-values for aroma were 0.003 for both groups, while for taste they were 0.003 (control) and 0.001 (experimental). Texture p-values were 0.009 (control) and 0.000 (experimental), and for colour both groups had a p-value of 0.000. In contrast, overall acceptability was normally distributed in both groups, with p-values of 0.402 (control) and 0.883 (experimental). These findings imply the necessity of using non-parametric statistical tests for analysis of aroma, taste, texture, and colour data, while parametric tests can be applied to overall acceptability. Consequently, the data were not considered to be fully normally distributed, necessitating the use of the Mann–Whitney U test as an alternative to the independent t-test to determine significant differences between formulations.

Treatment of products was tested by frying sausages in both product formulations. Each product sample, measuring 3 cm in length and 19 mm in width, is characterized by the following features: a smooth surface texture, vibrant colour indicative of its ingredients, consistent weight for uniformity, and a balanced flavour profile that appeals to a diverse consumer base.

Figure 2 presents a comprehensive sensory evaluation of four product samples (A1, A2, B1, and B2) across five key attributes: aroma, taste, texture, colour, and overall impression. The data include mean scores and standard deviations for each attribute, providing insights into the performance and consistency of the samples.

Sample A2 stands out as the top performer, achieving the highest mean scores in aroma (3.88 ± 0.83), taste (3.72 ± 0.98), and overall impression (3.7 ± 0.82). In contrast, sample B1 consistently receives the lowest ratings, particularly struggling in the taste category with a mean score of 2.72 ± 1.03. However, B1 unexpectedly excels in colour, with the highest mean score of 3.8 ± 0.92 among all samples. Samples A1 and B2 generally fall between the performance extremes of A2 and B1, with B2 showing slightly stronger overall ratings.

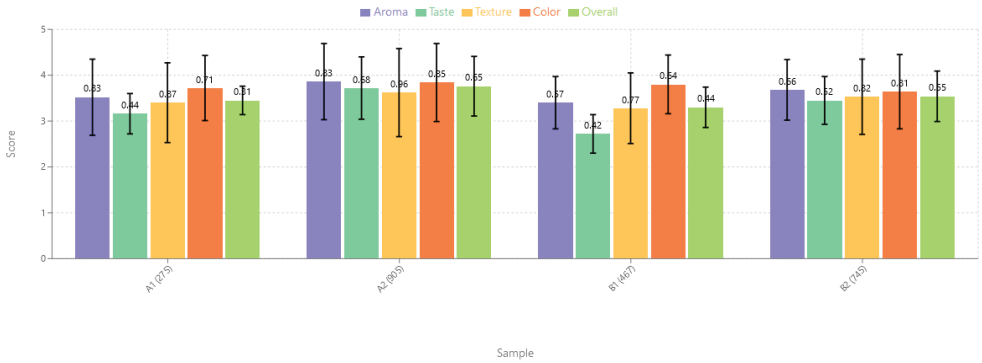

Figure 2. Results of the organoleptic test of the quality of white oyster mushroom sausage

Interestingly, the colour attribute exhibits the least variation in mean scores across samples, while taste shows the widest range and generally high standard deviations (0.98 to 1.23), suggesting diverse consumer responses. Sample B2 often demonstrates the lowest standard deviations, such as 0.75 for both aroma and texture, indicating more consistent evaluations. In contrast, B1 shows high variation in several attributes, with standard deviations ranging from 0.92 to 1.03. The overall scores align well with the patterns observed in the individual attributes, with A2 leading (3.7 ± 0.82) and B1 trailing (3.3 ± 0.98). This nuanced dataset highlights the strengths and areas for improvement of each sample, providing valuable insights for product development and optimization. The standard deviations offer additional information about the consistency of panellist responses, which can guide further refinements and targeted improvements in the sensory profiles of the samples.

Aroma plays a crucial role in the overall sensory experience of food products, significantly influencing consumer preference and acceptance (*Berčík et al.*, 2023). Within the food industry, understanding and optimizing aroma profiles are essential for developing successful products. In this study, the aroma of soy protein isolates in vegetarian sausages incorporating white oyster mushrooms was evaluated. The distribution of aroma scores, based on the average of two replicates, is presented in *Figure 2* (a. aroma).

Table 2. Hedonic data and Mann–Whitney results of the aroma indicator

Parameters	Control		Experiment		Significance 5%
	A1	A2	B1	B2	
Aroma	3.52	3.88	3.40	3.68	Not significant
Mean	3.7		3.54		
Acceptability					
Asymp. sig. (2-tailed)			0.496		
α			0.05		
Conclusion	*Asymp. sig. (2-tailed)* ≥ *Alpha*, no significant difference between control and experimental formulations				

The analysis in *Table 2* reveals no significant difference in aroma between control and experimental sausage formulations (asymp. sig. (2-tailed) = 0.496, $p > 0.05$). However, a subtle difference was noted: the experimental formulation, containing soy protein isolate, exhibited a stronger soy aroma compared to the control. This aligns with the mean favourability scores from *Table 2*, where the control (3.7, "like") slightly exceeded the experimental (3.54, "like"). This observation is consistent with prior research by *Flores & Piornos* (2021), who demonstrated that sausage aroma is influenced by both spices and binders. In our study, the increased soy protein isolate content in the experimental formulation likely contributed to the slightly lower aroma favourability.

Table 3. Hedonic data and Mann–Whitney U test results of taste indicators

Parameters	Control		Experiment		Significance 5%
	A1	A2	B1	B2	
Taste	3.16	3.72	2.73	3.40	Not significant
Mean	3.44		3.06		
Acceptability					
Asymp. sig. (2-tailed)			0.075		
α			0.05		
Conclusion	*Asymp. sig. (2-tailed)* > *Alpha*, no significant difference between control and experimental formulations				

The Mann–Whitney U test results presented in *Table 3* indicate no significant difference in taste between the control and experimental sausage formulations (asymp. sig. (2-tailed) = 0.075, $p > 0.05$). Although not statistically significant, a slight difference in mean favourability scores was observed, with the control

formulation (3.44, "quite like") rating higher than the experimental formulation (3.06, "quite like"). This trend may be attributed to the addition of soy protein isolate in the experimental formulation, which could impart a slightly bitter taste, as reported in a similar study by *Maya et al.* (2023).

The taste of sausages can be influenced by various factors, including fillers, binders, and seasonings, with soy protein isolate potentially contributing to a subtle decrease in taste acceptability in this case.

Texture is a property resulting from a combination of several physical properties, consisting of food shape, size, amount, and material-forming elements that can be felt by the senses of touch and taste, as well as through the mouth and eyes. Food products are made not only to increase nutritional value but to obtain characteristics that suit the organoleptic tests of consumers (*Guiné et al.*, 2020).

Table 4. Hedonic data and Mann–Whitney U test results of texture indicators

Parameters	**Control**		**Experiment**		**Significance** 5%
	A1	A2	B1	B2	
Texture	3.40	3.44	3.28	3.44	Not significant
Mean	3.42		3.36		
Acceptability					
Asymp. sig. (2-tailed)	0.782				
α	0.05				
Conclusion	*Asymp. sig. (2-tailed)* > *Alpha*, no significant difference between the control and experimental formulations				

The statistical analysis (Mann–Whitney U test) revealed no significant difference in texture between the control and experimental sausage formulations (asymp. sig. (2-tailed) = 0.782, $p > 0.05$). Although mean favourability scores for texture were slightly higher in the control (3.42, "quite like") compared to the experimental group (3.36, "quite like"), this difference was not statistically significant (*Table 4*). Both formulations exhibited a dense and chewy texture, attributed to the amylose and amylopectin content in the tapioca flour used as a binder. While soy protein isolate, added to the experimental formulation, is known to enhance emulsion properties in meat products (*Mazumder et al.*, 2023), it did not significantly alter the perceived texture in this study.

Colour is the initial sensory attribute perceived by consumers, and it plays a crucial role in shaping their expectations and perceptions of food quality. A product's colour, if it deviates significantly from what is expected for that type of food, can influence a panellist's assessment. Conversely, a colour that closely

resembles the natural or expected hue often creates a positive impression of quality and freshness (*Sipos et al.*, 2021).

Table 5. Hedonic data and Mann–Whitney U test results of colour indicators

Parameters	Control		Experiment		Significance 5%
	A1	A2	B1	B2	
Colour	3.72	3.76	3.80	3.64	Not significant
Mean	3.74		3.72		
Acceptability					
Asymp. sig. (2-tailed)	0.894				
α	0.05				
Conclusion	*Asymp. sig. (2-tailed)* > *Alpha*, no significant difference between control and experimental formulations				

The Mann–Whitney U test results in *Table 5* indicate no significant difference in colour between the control and experimental sausage formulations (asymp. sig. (2-tailed) = 0.894, $p > 0.05$). This aligns with the observed mean favourability scores, which were similar for both the control (3.74, "like") and experimental (3.72, "like") groups. The frying process, used for both formulations, resulted in a comparable brown colour. The addition of soy protein isolate in the experimental formulation did not appear to substantially alter the final colour of the sausages, consistent with findings in previous studies (*Kang et al.*, 2022; *Serdaroglu & Ozsumer*, 2003).

Overall acceptability represents the panellists' holistic evaluation of the white oyster mushroom vegetarian sausage enriched with soy protein isolate. It encompasses their integrated perception of all sensory attributes, including aroma, taste, texture, and colour, for each formulation. The hedonic assessment of overall acceptability serves as a crucial indicator of consumer liking, reflecting their comprehensive judgment of the product's sensory appeal (*Schouteten et al.*, 2018).

The statistical analysis (Mann–Whitney U test) revealed no significant difference in overall acceptability between the control and experimental sausage formulations (asymp. sig. (2-tailed) = 0.251, $p > 0.05$), as shown in *Table 6*. However, a subtle preference for the control formulation (mean = 3.57, "like") was observed compared to the experimental formulation (mean = 3.42, "quite like"). Panellists noted a stronger soy aroma and taste in the experimental sausages containing 20% soy protein isolate, which may have influenced their overall preference. Additionally, the texture and colour, also affected by the soy protein content, contributed to the panellists' overall assessment.

Table 6. Hedonic data and Mann–Whitney U test results of the overall indicator

Parameters	Control		Experimental		Significance 5%
	A1	A2	B1	B2	
Overall acceptability	3.45	3.70	3.30	3.54	Not significant
Mean	3.57		3.42		
Acceptance parameters					
Asymp. sig. (2-tailed)	0.251				
α	0.05				
Conclusion	*Asymp. sig. (2-tailed)* > *Alpha*, no significant difference between control and experimental formulations				

These findings suggest that while the addition of soy protein isolate did not significantly alter the overall acceptability of the vegetarian sausages, it did introduce subtle sensory changes that some panellists found less desirable compared to the control. This aligns with the research by *Sari et al.* (2021), who reported that sausages with lower concentrations of soy protein isolate, closer to the control, were generally preferred.

The price of vegetarian sausages per serving with a length of 6 cm and 19 mm has a selling price of 1,088 IDR, and the selling price of white oyster mushroom vegetarian sausages with the addition of soy protein isolate has a selling price of 1,235 IDR. White oyster mushroom vegetarian sausage with the addition of soy protein isolate has a slightly more expensive selling price compared to white oyster mushroom vegetarian sausage. However, the selling price of vegetarian sausages is cheap because the price of white oyster mushrooms and soy protein isolate is cheaper than meat. Making sausages with ingredients including white oyster mushrooms can reduce the selling price as compared to cases using meat (*Mazumder et al.*, 2023).

Table 7. Results of proximate chemical analysis

Composition	Control sausage (%)	Experimental sausage (%)
Moisture	65.00 ± 0.23^{a}	63.50 ± 0.19^{b}
Protein	0.42 ± 0.04^{a}	2.24 ± 0.05^{b}
Fat	2.28 ± 0.08^{a}	2.26 ± 0.07^{b}
Ash	2.15 ± 0.02^{a}	2.27 ± 0.03^{b}
Carbohydrate	30.15 ± 0.35^{a}	29.73 ± 0.34^{b}

Note: Numbers followed by the same letter in the same row indicate no significant difference at the 5% significance level.

The chemical analysis results presented in *Table 7* demonstrate statistically significant differences between the control and experimental samples across all measured parameters. This conclusion is supported using superscripts *a* and *b*, which indicate that the Mann–Whitney U test revealed significant differences at the 5% level for moisture, protein, fat, ash, and carbohydrate content. The experimental sample showed a notable increase in protein content (from 0.42% to 2.24%) and slight decreases in moisture (from 65.00% to 63.50%) and carbohydrate content (from 30.15% to 29.73%). Meanwhile, fat and ash content remained relatively stable with minor increases in the experimental sample. These consistent statistical differences across all parameters suggest that the experimental treatment had a substantial and measurable impact on the product's composition. However, it is important to note that despite these significant changes, particularly in protein content, the experimental sample still falls short of meeting the minimum protein requirements set by the SNI standard for meat sausage. This indicates that while the experimental treatment resulted in meaningful compositional changes, further modifications would be necessary to fully comply with regulatory standards for meat sausage products.

Based on the SNI 3820:2015 standard for meat sausage, both the control and experimental samples show mixed compliance with the requirements. While they meet the standards for fat content (max. 20%), moisture content (max. 67%), and ash content (max. 3.0%), they fall significantly short in protein content. The standard requires a minimum of 13% protein for meat sausage and 8% for combined meat sausage, but the control sample contains only 0.42% protein and the experimental sample 2.24%. This is a critical deficiency, as protein is a key nutritional component in meat products. The slight reduction in moisture content in the experimental sample (63.50% vs 65.00% in the control) is within the allowable range and could potentially contribute to a longer shelf life. The marginal increase in ash content in the experimental sample (2.27% vs 2.15%) is also within limits and suggests a slightly higher mineral content. There is no specific requirement for carbohydrate content in the SNI standard, so the slight decrease observed in the experimental sample (29.73% vs 30.15%) is not a compliance issue. To meet the SNI Standard and be classified as either meat sausage or combined meat sausage, significant product reformulation would be necessary to substantially increase the protein content while maintaining the current compliance in other areas.

Statistical analysis using the Mann–Whitney U test at the 5% significance level indicated no significant differences between control and experimental groups for any measured components. While the samples appear to meet SNI standards for fat and ash content and exceed requirements for carbohydrates, the protein levels are notably below the required minimum. This suggests a critical need for reformulation to increase protein content to meet the SNI 01-3820:1995 standard for sausage products. Future studies should report results in both percentages and grams to facilitate direct comparison with SNI standards.

Table 8. Results of microbial analysis

Analysis type	Experimental sausage	Control sausage	SNI 3820:2015	Conclusions
Total Plate Count (TPC)	7.4×10^2 CFU/g	1.3×10^3 CFU/g	max. 10^5 CFU/g	Safe for consumption
Coliform test	< 3 MPN/g	< 3 MPN/g	max. 10 MPN/g	Safe for consumption
Salmonella sp.	absent	absent	absent in 25 g	Safe for consumption

The microbial test results presented in *Table 8* provide a comprehensive analysis of the safety of both experimental and control sausage samples, evaluated against the Indonesian National Standard 3820:2015 for sausage products (*Badan Standardisasi Nasional*, 2015).

Three key microbial parameters were assessed: Total Plate Count (TPC), coliform presence, and *Salmonella* sp. detection, which are standard indicators of food safety and hygiene (*Arroyo-López et al.*, 2014). The experimental sausage showed a TPC of 7.4×10^2 CFU/g, while the control sausage had a slightly higher count at 1.3×10^3 CFU/g, both being well below the maximum allowable limit of 10^5 CFU/g.

For coliform determination, both samples registered less than 3 MPN/g, comfortably under the standard's maximum of 10 MPN/g. Additionally, *Salmonella* sp. was absent in both sausage types, meeting the requirement of absence in 25 g of sample. These results collectively indicate that both the experimental and control sausages are safe for consumption, successfully passing all three microbial safety criteria (*FDA*, 2001). Notably, the experimental sausage demonstrated a marginally lower bacterial count in the TPC test, suggesting that the experimental production method maintains, if not slightly improves, microbial safety standards compared to the control method. This aligns with recent studies indicating that innovative processing techniques can enhance food safety in meat products (*Zhang et al.*, 2010). Overall, these findings affirm that both sausage samples align with the established food safety regulations and are suitable for human consumption, reflecting adherence to good manufacturing practices in sausage production (*Heinz & Hautzinger*, 2007).

4. Conclusions

This study investigated the utilization of white oyster mushrooms (*Pleurotus ostreatus*) and soy protein isolate in the development of vegetarian sausages. Two formulations were evaluated: a control containing 100% white oyster mushrooms and an experimental formulation with 80% white oyster mushrooms and 20%

soy protein isolate. Sensory evaluation using a 5-point hedonic scale revealed no significant differences in aroma, taste, texture, or colour between the control and experimental sausages. The experimental formulation showed promising overall acceptability.

The chemical analysis revealed that the experimental formulation achieved a protein content of 2.24%, a significant increase compared to the control's 0.42%. However, both values fell short of the Indonesian National Standard (SNI 3820:2015), which mandates a minimum protein content of 8% for combined meat sausages. The moisture, fat, and ash levels were consistent with SNI guidelines, and the slight variations observed did not impact compliance. Additionally, microbiological assessments confirmed that both formulations adhered to safety standards, with TPC, coliform counts, and absence of *Salmonella* meeting SNI requirements.

In conclusion, white oyster mushroom vegetarian sausages enriched with soy protein isolate present a nutritionally and economically promising plant-based alternative to conventional meat products. The incorporation of soy protein isolates significantly enhanced the protein content without compromising sensory attributes or microbiological safety. This research underscores the potential of utilizing mushrooms and plant proteins in developing sustainable, healthy, and affordable meat substitutes.

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How to feed your microbiome? The role of dietary fibres in shaping microbiome and host health

K. I. Ilkei[1,2]
e-mail: ilkei.klementina@gmail.com

S. Jakab-Kodácz[3]
e-mail: jakabkodaczstefania@gmail.com

Gy. Mara[2]
e-mail: maragyongyver@uni.sapientia.ro
ORCID: https://orcid.org/0000-0001-8836-069X
[1] Faculty of Science, Doctoral School of Chemistry,
University of Pécs, Vasvári Pál Street 4, 7622 Pécs, Hungary
[2] Sapientia Hungarian University of Transylvania,
Faculty of Economics, Socio-Human Sciences and Engineering,
Department of Bioengineering, RO-530104 Miercurea Ciuc, 1 Libertății Sq.
[3] Faculty of Science, Doctoral School of Biology,
University of Pécs, Vasvári Pál Street 4, 7622 Pécs, Hungary

Abstract. Feeding our microbiome involves understanding the complex interactions between diet and diverse microbial communities residing in the human gut. The gut microbiota plays a crucial role in maintaining health, and its composition can be influenced by dietary components such as dietary fibres. Therefore, this article presents knowledge on the relationship between different diets and the microbiome and focuses on the current understanding of dietary fibres and their role in shaping the microbiome. The reader will gather information about the role of the microbiome in health and disease status and the composition of the eubiotic and dysbiotic microbiota. Finally, this article focuses on microbial secondary metabolites, particularly short-chain fatty acids, with outstanding effects on health status.

Keywords and phrases: polysaccharides, oligosaccharides, prebiotic, eubiosis, dysbiosis, short-chain fatty acids

1. Introduction

The human microbiome is defined as a characteristic microbial community (viruses, bacteria, and fungi) residing in the human body. The aim of this article is to provide an overview of the current understanding of the role of nutritional fibres in shaping the microbiome and the role of the microbiome in health maintenance.

The question of how to feed our microbiome has become a burning issue today, both in the social and scientific areas. Even though the role of dietary fibre (DF) was recognized in the 1950s, the way we approach the subject today has changed considerably due to its role in shaping the microbiome. Since 2016, when the Human Microbiome Project ended (*NIH Human Microbiome Project – About the Human Microbiome*, n. d., *Lloyd-Price et al.*, 2017), knowledge regarding the composition and role of the human microbiome in our health/disease status has increased enormously. This current knowledge rewrites our understanding of fibres and their role in shaping the microbiome. A healthy microbiome, through its secondary metabolites (short-chain fatty acids), helps maintain a healthy status (*Cronin et al.,* 2021). However, the complexity of these interactions poses challenges, and future perspectives in this field would be personalized dietary recommendations to optimize health outcomes.

2. Nutritional role of dietary fibres

To understand the nutritional role of dietary fibre, we need to look into its history. Hippocrates was the first to recognize the benefits of dietary fibre in 430 BC; unfortunately, this discovery was forgotten at that time (*McBurney et al.*, 2019). As a term, DF first appeared in the 1950s. Initially, it was connected to substances derived from plant cells; later, it was specified that this meant plant-derived polysaccharides are indigestible by humans and cannot be absorbed through the gastrointestinal tract. Gradually, it was found that dietary fibres have more than just nutritional benefits (*Kshirsagar et al.*, 2020).

Modern thinking recognizes that the quality and type of DF influence the gut microbiome, thus affecting the health of the host (*McBurney et al.*, 2019). We know now that DF, consisting of polysaccharides, lignin, and oligosaccharides, helps maintain gut health by aiding digestion, reducing constipation, and other beneficial factors. DF support beneficial gut bacteria by serving as prebiotic substrates (*Timm & Slavin*, 2023). There are several other known health benefits, including lowering plasma lipid levels, stabilizing blood sugar levels, and reducing inflammation. We can also mention protection against diseases such as stroke, type 2 diabetes, and even cancer (*Kim & Je*, 2014). However, they are important in regulating appetite, and studies have shown their role in increasing satiation and reducing hunger, resulting in reduced energy intake and weight loss (*Akhlaghi*, 2024).

In addition to dietary fibre, other nutrients are also important. Because nutrition recommendations are related to calorie intake, they differ between sexes and age classes. The recommended daily intake of dietary fibre is 38 g/day for healthy men, and 25 grams g/day for healthy women (*Slavin*, 2005). The recommendations of Nordic Nutrition in 2012 for healthy people are similar to the previously mentioned

amounts, more precisely the same for women and 35 g/day for men (*Carlsen & Pajari*, 2023). According to the United States Institute of Medicine, the current recommended daily dietary fibre consumption for different age classes ranges from 14 to 20 g/day for children, 22–30 g/day for adolescents, and 25–38 g/day for the elderly (*Yusuf et al.*, 2022).

Diet and eating habits have a major impact not only on health but also on the quality of life of the host, which is influenced by geographical, religious, ethical, and cultural factors. Today, one of the most recognized diets is the Mediterranean diet, which is popular for its health benefits such as reducing the risk of infectious diseases. The diet focuses on the consumption of vegetables, fruits, whole grains, nuts, low dairy consumption, moderate fish consumption, and unsaturated fats such as olive oil (*Klement & Pazienza*, 2019). Similarities can be drawn between a vegetarian diet, which typically avoids meat, poultry, and fish, and a vegan diet, which avoids all animal products (*Key et al.*, 2006).

Overall, we could say that despite the initial recognition, which proved difficult, and that the thinking of the time ignored Hippocrates's observations on the physiological effects of dietary fibre (*McBurney et al.*, 2019), today it is becoming an important and essential part of understanding more both personalized diets and research topics.

3. Classification of dietary fibres

Dietary fibres can be identified as carbohydrate polymers and oligomers, which are comprised of sugar units such as glucose, fructose, galactose, xylose, and arabinose (*Cantu-Jungles et al.*, 2021). The main part of plants that has an impact on health is their cell walls. These supramolecular cell walls mostly contain cellulose, hemicelluloses, pectin, and lignin (*Augustin et al.*, 2020). The overly simplistic method for classifying dietary fibres is either soluble or insoluble. This kind of comparison comes from its physicochemical properties, based on the fibre content analysis of foods (*Puhlmann & de Vos*, 2022). One of the most studied soluble fibres in this field is inulin-type fructooligosaccharides (FOS). Additionally, some new studies have also proven that insoluble fibres, such as chitin-β-1,3 glucan, can have a huge impact on the intestinal tract (altering the *Clostridium* cluster) (*Cantu-Jungles et al.*, 2018). However, the physicochemical characterization of fibres is limited. To gain a full understanding of dietary fibre, we must also analyse its functional properties.

The fibres that we consume have specificity, which describes how many bacterial strains are able to use each of them for their life cycle (*Puhlmann & de Vos*, 2022). Dietary fibres can be distinguished based on their low or high specificity (*Figure 1*). *Cantu-Jungles et al.* evaluated the microbiota fermentation of fibres,

classifying each with low to high specificity. Dietary fibres with low specificity are fructooligosaccharides because of their simple chemical structure and are often used in the Western diet. Fibres with low-to-intermediate specificity, such as type II resistant starch, also have simple structures and are common in most diets, but they are insoluble in water, which increases their specificity. Pectin belongs to the intermediate specificity group because it has a complex chemical structure. However, it is soluble in water; therefore, it does not belong to the high-specificity group. β-glucan fibres have high specificity because they are insoluble in water and are uncommon in our diet. It has been proven that if we have a specific bacterial strain belonging to the *Anaerostipes* genus or *Bacteroides uniformis*, these communities can be sustained by consuming high-specificity fibres such as β-glucan (*Cantu-Jungles et al.*, 2021). β-glucans are the most studied fibres in this field (*Rahman et al.*, 2023). Overall, eating high-specificity dietary fibres can cause a dramatic shift in the microbial community of the gut (*Cantu-Jungles et al.*, 2021).

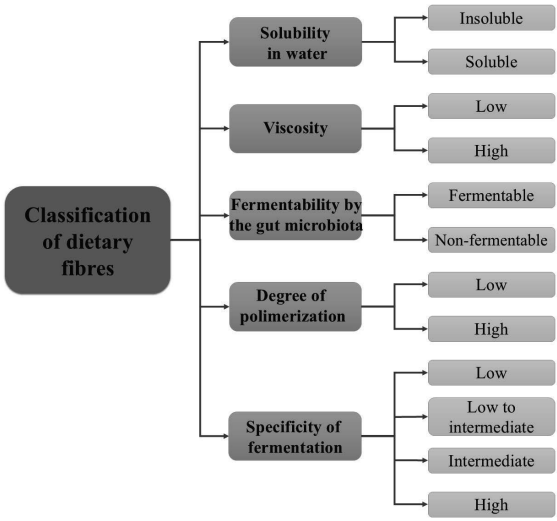

Figure 1. The classification of dietary fibres based on their properties

Dietary fibres can also be differentiated based on their fermentability and viscosity. Whether a fibre is fermentative (inulin and fructooligosaccharides) or non-fermentative (cellulose) depends on whether the bacterial community in the gut can metabolize it. We can say that a fibre is fermentable if the bacteria in the proximal gastrointestinal tract can metabolize it; otherwise, they are non-fermentable (*Rahman et al., 2023*). We can also characterize soluble fibres based on their viscosities. Viscosity is a physicochemical feature, which indicates how resistant the fibre is against the flow we are talking about. This also means how

thick or jelly-like will the fibre be after mixing with aqueous solutions (*Rahman et al.*, 2023). If some dietary fibres are mixed with aqueous solutions, they can form a gel, which means that they have gel-forming capacity. The concentration, ionic type, temperature, and chemical composition influence gel-forming capacity *(Cui et al., 2013*). All fibres have different physicochemical features, impacting the body and human health differently through the gut microbiome (*Rahman et al.*, 2023).

The final characterization method is to determine the molecular weight of the fibre. We can distinguish whether they have a high or low molecular weight. Non-starch polysaccharides, resistant starch, and other associated substances, such as lignin, have high molecular weights. Oligosaccharides and inulin have low molecular weights based on their chemical structures.

The presence of dietary fibre in food can be determined using either the Englyst method (NSP) or the Association of Official Analytical Chemists method (AOAC). The soluble and insoluble dietary fibre concentrations of the different foods was determined by *Li et al.* (2002).

Dietary fibre is often used as a synonym for prebiotic substances. *Rezende et al.* classified dietary fibres as prebiotics, candidates for prebiotics, and not recognized prebiotics. Prebiotics should beneficially affect the host by selectively stimulating the growth and metabolic activity of beneficial gut bacteria. Fructooligosaccharides and galactooligosaccharides are considered in literature as prebiotics, fermentable carbohydrates as mannanoligosaccharides, and xylooligosaccharides as prebiotic candidates – based on *in vitro* and preclinical studies (*Rezende et al.*, 2021).

4. Healthy and dysbiotic microbiome

The human microbiome, a complex ecosystem of microorganisms, plays a crucial role in maintaining health and contributes to disease when imbalanced. The human microbiome consists of diverse microorganisms, including bacteria, fungi, archaea, protozoa, and viruses, which are abundant in different organ systems such as the digestive, respiratory, urinary, reproductive, and neural systems (*Link*, 2021; *Xiao et al.*, 2024). The impact of microbiota on health and various diseases has been known since the 17th century. A healthy microbiome, also known as eubiosis, supports metabolic functions, immune modulation, and protection against pathogens. In contrast, dysbiosis, which is an imbalance in microbial communities, is associated with various diseases. In the mid-2000s, progress in modern sequencing technologies and metagenomics made it possible to study the composition and function of the microbiome in depth (*Bresalier & Chapkin*, 2020).

Microbial colonization begins after birth and undergoes gradual transformation. Many influencing factors can affect colonization. These factors include ethnicity, geographical location, economic status, and social status, all of which contribute

to the formation and development of this bacterial community. The most important factors influencing the microbiome during pregnancy are the maternal microbiome, mode of delivery (vaginal babies have a more diverse gut microbiota in the first two years than those born by caesarean section), diet, antibiotics, and probiotics. Microbial colonization in infancy undergoes the greatest changes during the first few years of life, and thereafter shows relative stability and constancy depending on environmental factors (*McBurney et al.*, 2019).

These microbes perform essential functions such as aiding digestion, producing vitamins, and protecting against infections. In particular, gut microbiota is crucial for metabolism and immune system activation, influencing the central nervous system and contributing to overall health *(Haripriya et al.*, 2024*; Marano et al.*, 2023). The composition and function of the microbiome, particularly the gut microbiome, dynamically change in response to the actual diet of the host, antibiotic use, and other environmental factors, and resilience is an important characteristic of a healthy microbiome, that is, the ability to return to a state of equilibrium (*Bresalier & Chapkin*, 2020).

The human microbiome is composed of several key phyla, including *Firmicutes*, *Bacteroidetes*, *Actinobacteria*, and *Proteobacteria*. These phyla interact intricately with each other and with the host, thereby influencing health and disease states. *Firmicutes* and *Bacteroidetes* are the dominant phyla in the human gut microbiome. *Firmicutes* are involved in energy metabolism and are known for their role in breaking down complex carbohydrates, whereas *Bacteroidetes* are crucial for the degradation of proteins and polysaccharides (*Ravikrishnan & Raman*, 2021; *Zhang et al.*, 2023). *Actinobacteria*, including *Bifidobacterium*, are important for maintaining gut health and have been associated with anti-inflammatory properties (*Yang et al.*, 2023). *Proteobacteria* are less abundant, include many pathogenic species, and are often associated with dysbiosis and inflammatory conditions (*Ravikrishnan & Raman*, 2021). Microbiota plays a crucial role in an individual's health, and a reduction in its diversity can increase the risk of developing certain diseases.

A diet rich in salt, low in fibre, carbohydrate-oriented, and lacking in physical activity negatively affects the microbiome, which may be associated with the potential development of certain chronic diseases later in life (*McBurney et al.*, 2019). For example, growing evidence suggests that the gut microbiome plays a significant role in the development and progression of colorectal cancer (CRC). CRC is the second leading cause of cancer-related deaths. Increased numbers of *Fusobacterium nucleatum* have been detected in stool samples and tumour tissues of patients with the disease (*Zhang et al.*, 2023). In addition, an imbalance in the gut microbiota, known as dysbiosis, has been associated with weight gain and low levels of inflammation, which impair glucose metabolism. Soluble fibres, such as oligofructose and long-chain inulin, can help restore the balance of gut

flora, reduce weight gain, and improve glucose metabolism (*Makki et al.*, 2018). Although the associations between different dietary components and health or disease are strong, further research is required to identify microorganisms with proven therapeutic potential (*Wilson et al.*, 2020).

5. The difference in microbiome composition caused by low and high fibre intake

Dietary fibre consumption is particularly important in shaping the composition of the microbiome. High fibre intake generally promotes a more diverse and beneficial microbiome, whereas low fibre intake can lead to less favourable microbial profiles. The health benefits of dietary fibre are mediated by gut microbiota, as fibre fermentation in the colon promotes the growth of beneficial bacteria, which produce beneficial metabolites, lower pH levels, and inhibit harmful bacteria. High-fibre diets promote an increase in beneficial bacteria, such as *Bacteroides* and *Bifidobacterium*, which are linked to improved immune responses and reduced tumour-promoting genera (*Sharma et al.*, 2024). Inadequate or insufficient fibre intake (which is common in industrialized nations) alters the microbial composition of the gut, which can lead to the development of many chronic diseases *(Kok et al.*, 2023). Low-fibre diets can reduce the abundance of beneficial bacteria and alter the production of regulatory immune molecules, such as IL-10, which is crucial for maintaining intestinal homeostasis *(Rivera-Rodriguez et al.*, 2023). Fibre from fruits and vegetables helps to maintain microbiota diversity, positively influences its structure and function, and leads to the production of short-chain fatty acids (SCFAs) through fermentation (*Tanes et al.*, 2021). Understanding how dietary fibre affects the gut microbiota can be key to the battling of chronic diseases (*Oliver et al.*, 2021).

The individual variability of the gut microbiome responds accordingly to different dietary fibres, which is why personalized diets are becoming increasingly important and popular in improving gut health and metabolic efficiency (*Kok et al.*, 2023). The Western diet, high in red meat, saturated fats, processed grains, and added sugars and low in fruits, fibre, vegetables, nuts, whole grains, and seeds, has a distinct impact on the gut microbiome. Studies have shown that it leads to lower levels of beneficial bacteria, such as *Prevotella copri*, and higher levels of species associated with negative health condition such as *Alistipes* and *Ruminococcus* (*Dahl et al.*, 2020). The Mediterranean diet (consumed in Mediterranean countries such as Spain, Greece, and Italy) is characterized by plant-based foods (fruits and vegetables, whole grains), olive oil, moderate fish and poultry intake, limited dairy, and a joyful, active lifestyle. Research shows that this

diet positively influences gut microbiota, particularly boosting beneficial bacteria such as *Faecalibacterium prausnitzii*, which produces butyrate, and reducing potentially harmful bacteria such as *Ruminococcus gnavus*. Additionally, lower adherence to this diet is linked to higher levels of urinary trimethylamine-N-oxide (TMAO), a compound associated with cardiovascular and other chronic diseases (dementia and hypertension), which may signal the development of insulin resistance. These findings suggest that the Mediterranean diet may reduce disease risk through its effect on gut health (*Dahl et al.*, 2020). The typical American diet is low in fibre, with less than 10% of Americans meeting the recommended daily fibre intake. In contrast, most, although not all, vegetarians and vegans consume higher amounts of dietary fibre (*Tanes et al.*, 2021). Vegetarian and vegan diets are often considered beneficial for health because of their higher intake of plant-based foods, which typically results in greater fibre consumption. Studies have found variations in specific microbiota components, such as reduced levels of *Collinsella* and *Holdemania*, but increased levels of *Roseburia* and *Lachnospiraceae* in vegetarians. Plant-based diets influence microbiota function, including higher microbial gene and protein abundance involved in polysaccharide and protein breakdown, and vitamin synthesis (*Kok et al.*, 2023). Human studies involving controlled diets have demonstrated that vegetables high in inulin can increase the levels of *Bifidobacterium*, promote a feeling of fullness, and aid in weight reduction (*Armet et al.*, 2022). A ketogenic diet, which is characterized by fats and is very low in carbohydrates, induces body ketosis and relies on fat for energy. Research on this diet has shown varying effects on gut microbiota, including reduced *Firmicutes* and increased *Bacteroidetes*. Some findings have indicated that a ketogenic diet may lead to decreased microbiome diversity. Furthermore, a modified Mediterranean-ketogenic diet has been associated with changes in microbiota composition and a reduction in Alzheimer's disease biomarkers in the cerebrospinal fluid (*Dahl et al.*, 2020). The Paleolithic diet imitates the eating habits of ancient humans, emphasizing meats, fish, nuts, seeds, healthy oils, fresh fruits, and vegetables, while avoiding legumes, dairy, grains, refined sugars, and processed foods. Research has indicated that this diet supports a diverse microbiome (*Dahl et al.*, 2020).

There is growing evidence that microbial responses are individualized. When comparing dietary fibre studies, it is important to consider all the aspects. For example, in a group of obese patients, different changes in body mass index (BMI) were noted after three months of inulin supplementation. Responders who experienced a decrease in BMI had higher baseline levels of *Akkermansia* and *Butyricicoccus* and lower levels of *Anaerostipes*. Responders in this group showed increased levels of *Prevotella copri* and genes related to β-glucan degradation, which in subsequent mouse studies were linked to greater glycogen storage in the liver (*Kok et al.*, 2023). In murine models on a high-fat diet, the benefits of

inulin, including promotion of the incretin hormone glucagon-like peptide 1 and protection against metabolic syndrome, were diminished when antibiotics were used, suggesting a role for the gut microbiota (*Armet et al.*, 2022).

Studies on the importance of dietary fibre and the microbiome emphasize the complexity and variability of the gut microbiome, which can result in significant differences in individual responses to dietary interventions (*Oliver et al.*, 2021).

6. Metabolic function of gut microbiome

The gut microbiota is a "living metabolic organ" that can interact with the human body and influence its state of health. Human hosts can use these metabolites as energy sources (*Schippa & Conte*, 2014). The microbiota can produce many metabolites, including vitamins (*Soto-Martin et al.*, 2020) and short-chain fatty acids (SCFA) (*Fusco et al.*, 2023), influence ion absorption (*Engevik & Engevik*, 2021), and synthesise amino acids (*Ashniev et al.*, 2022).

In the intestinal tract, we can distinguish microbial-accessible carbohydrates (MACs), which are complex polysaccharides and oligosaccharides that the gut microbiome can degrade by carbohydrate-active enzymes (CAZymes). CAZymes are microbial enzymes for degrading endogenous and exogenous, simple to complex carbohydrates with high specificity. Since 1999, the Carbohydrate-Active Enzymes database (CAZy; http://www.cazy.org) coupled with the CAZypedia encyclopedic resource has been available, which offers updated classification data of CAZymes (*Lombard et al.*, 2014). *Bacteroidetes* can degrade different kinds of polysaccharides with the help of glycan-binding proteins because they have polysaccharide utilization loci (PUL), which encode the degrading system for them (*Cheng et al.*, 2022). *Firmicutes*, *Roseburia* spp., and *Eubacterium rectale* possess carbohydrate-active enzymes (CAZymes) *(Sheridan et al.*, 2016) and are crucial members of the metabolism of fibre substrates in the gut microbiome (*Kok et al.*, 2023). They specialize in the use of different carbohydrate substrates. The *Roseburia/E. rectale* group can utilize starch and fructooligosaccharides. However, not all members of *Roseburia* genus are capable of completely using these dietary fibres; for example, *R. hominis* is less capable (*Sheridan et al.*, 2016). *Proteobacteria* is one of the most abundant phyla in the human gut (*Rizzatti et al.*, 2017). In 2018, *Méndez-Salazar et al.* proved that a relatively high abundance of phyla can predict gut dysbiosis (*Méndez-Salazar et al.*, 2018). However, many bacteria in the gut are anaerobic, and members of the *Proteobacteria* phyla are facultative or obligate anaerobes in humans and other mammals. Therefore, they also use oxygen in their life cycle, which lowers the redox potential in the gut. They also produce a relatively high range of metabolites (*Moon et al.*, 2018).

The microbial community can also produce bioactive polyphenols from dietary substrates such as fruits, vegetables, and cereals. The five-carbon and six-carbon monosaccharides can also be degraded by the classical pentose phosphate pathway or the Embden–Meyerhof–Parnas pathway (*Krautkramer et al.*, 2021).

7. The role of dietary fibres in microbial SCFAs production and host health

Dietary fibres affect the microbes of the gastrointestinal tract and the metabolic profile of the bacterial community. Several studies have shown that consuming dietary fibre has a positive effect on host health through the stabilization of blood glucose concentration, reduction of cholesterol levels, and gastrointestinal tract disorders (*Williams et al.*, 2017). Short-chain fatty acids (SCFAs) are produced by the gut microbiome as a result of dietary fibre fermentation. These organic compounds cannot be produced by human enzymes and can only be metabolized by specific microbes, mainly bacteria in the gut (*Fusco et al.*, 2023). The average SCFAs produced in the colon were 40 mM acetate, 15 mM propionate, and butyrate for a normal adult. These numbers are lowered if the individuals follow a Western diet and are raised by consuming a high dietary fibre diet (*Tan et al.*, 2023). As shown in *Figure 2*, SCFA molecules can affect health state, mood, and behaviour (*Fusco et al.*, 2023). However, most studies have shown that SCFAs have a significant positive impact on human health. Some studies have demonstrated side effects of SCFA production such as increasing hepatic lipid accumulation or inducing brain neurochemistry impairment (*Xiong et al.*, 2022).

In this section, we discuss different SCFA profiles based on dietary fibre consumption. *Chen et al.* published a study about the effect of arabinogalactan on the gut microbiome. They proved that supplementation of arabinogalactan by 30 healthy adults at 15 g each day decreased the level of isovaleric, valeric, and hexanoic acid production (*Chen et al.*, 2021). In 2012, *Damen et al.* investigated the effects of eating bread containing arabinoxylan oligosaccharides (AXOS). AXOS is the main dietary fibre component of cereals. In their study, they examined 27 volunteers aged between 18 and 46 years old. They showed that the consumption of *in situ* baked bread can increase butyrate concentration in faeces, and they also measured increased levels of acetate and propionate – but not significantly (*Damen et al.*, 2012). However, the effect of inulin on the gut microbiome remains unclear. Several studies have demonstrated changes in the SCFA profile after the daily utilization of inulin, but these changes were not significant. For example, *Reimer et al.* compared 50 healthy adults with low dietary fibre consumption (< 15 g/day in women, < 18 g/day in men) in their study. They examined subjects'

SCFA profiles based on inulin intake. The subjects were categorized into two groups. The first group consumed 7 g/day, and the second group consumed 3 g/day. There were no significant differences between the two groups compared to the placebo groups. However, some significant changes were detected in the faecal butyrate concentration, but after including the baseline concentration, the significance was eliminated (*Reimer et al.*, 2020). In their systematic review, *Vinelli et al.* analysed 42 studies on the effects of dietary fibre on SCFA production in the gut microbiome. From these 42 studies, seven demonstrated that dietary fibre consumption can significantly increase SCFA production (*Vinelli et al.*, 2022). Dietary fibre can also protect the intestinal barrier. The gut microbiome degrades several types of dietary fibres and produces butyrate, which can be an energy source for enterocytes. Enterocytes help to maintain the integrity of the epithelium (*Zhang et al.*, 2022).

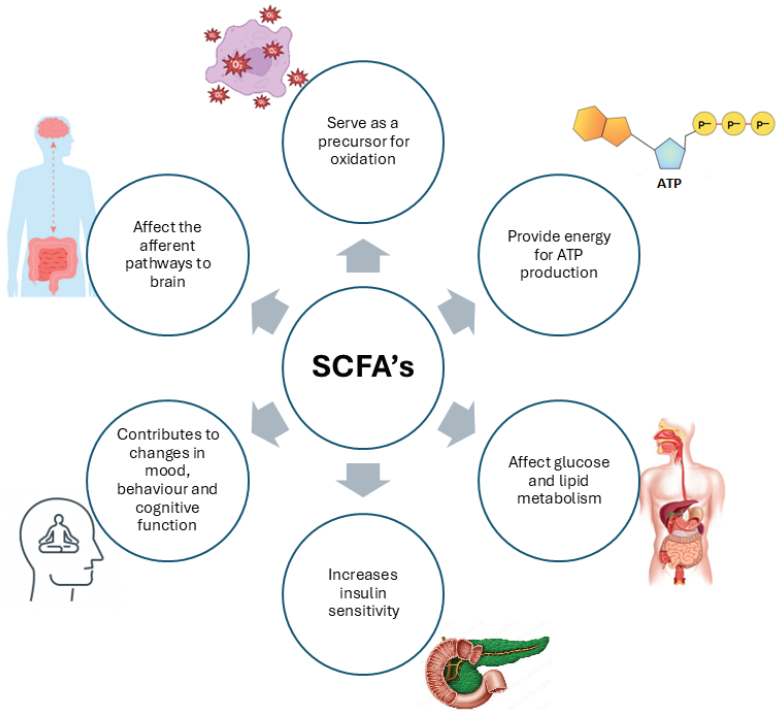

Figure 2. The benefits of SCFA molecules for host health

In 2021, *Medawar et al.* published an article that compared healthy and overweight adults. They examined the subjects' eating behaviour, BMI, microbiome composition, and SCFA profile from serum and faeces. Their results showed that

higher dietary fibre intake was significantly associated with lower body fat mass. In addition, they could correlate participants eating less with higher faecal propionate levels. In addition, they demonstrated that patients with lower body fat mass had higher serum acetate and butyrate levels. Based on these results, they stated that dietary fibre intake may modify unfavourable microbiota genera and thus the SCFA profile (*Medawar et al.*, 2021).

Some studies have attempted to combine dietary fibres to determine their impact on host health. *Peng et al.* fed mice with four different types of dietary fibres (pectin 98%, cellulose 99%, type II resistant starch 98%, and fructooligosaccharides 98%) for four weeks. They created three types of testing groups: single dietary fibre, a mixture of two dietary fibres, and a mixture of multiple dietary fibres. The mixture of fructooligosaccharides and cellulose resulted in higher total acid, lactic acid, and butyric acid concentrations, as well as in one of the highest concentrations of propionic and acetic acid in the gut (*Peng et al.*, 2013).

8. Concluding remarks and future perspectives

There is an intricate relationship between diet and the gut microbiota to promote health and manage diseases. For example, through fibre fermentation, the gut microbiota produces SCFAs, which improve metabolism-related disease conditions and potentially reverse metabolic dysfunctions associated with low-fibre diets. Despite the well-documented benefits of dietary fibre, individual responses to fibre supplementation can vary. Understanding the specific interactions between different types of fibres and the microbiome is essential for optimizing health outcomes. Therefore, personalized dietary recommendations for shaping the microbiome are emerging. However, complex host–microbiome interaction networks and their metabolic impacts require further exploration. Cutting-edge technologies, such as engineered organoids, high-throughput cultivation methods, and microfluidic assays, have enhanced the effectiveness and precision of microbiome studies. These technological advancements, combined with an expanding molecular understanding of how gut microbes interact with nutrients, are poised to deepen our understanding of the dietary effects on host health and disease, paving the way for individualized nutritional strategies and treatments. As this field evolves, it is essential to combine data from these innovative techniques with functional metabolic profiles and biomarkers to create more accurate and reliable dietary guidelines. However, upcoming studies in nutrigenomics could shed light on the mechanisms by which prebiotics interact with human genes to affect health outcomes. This research may lead to tailored dietary guidelines that incorporate prebiotic supplements based on the unique genetic profiles of individuals. Continued research and technological

advancements are essential to fully realize the potential of personalized nutrition in shaping the microbiome to improve health outcomes.

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Preliminary study of lavender-flavoured beer production methods

M. Kelemen[1]
e-mail: markelemen24@yahoo.com

Cs. Albert[1]
e-mail: albertcsilla@uni.sapientia.ro
ORCID:
https://orcid.org/0000-0001-6882-6691

Cs. D. András[1]
andrascsaba@uni.sapientia.ro
ORCID:
https://orcid.org/0000-0001-5220-832X

R. V. Salamon[1]
salamonrozalia@uni.sapientia.ro
ORCID:
https://orcid.org/0000-0002-3497-6859

[1] Sapientia Hungarian University of Transylvania,
Faculty of Economics, Socio-Human Sciences and Engineering,
Department of Food Science, RO-530104 Miercurea Ciuc, 1 Libertății Sq.

Abstract. The aim of our research was to produce a lavender-flavoured beer belonging to the Blonde Ale category, which can have a positive physiological effect on the consumer. Two flavouring methods were investigated: the addition of lavender flowers (control) during hop boiling and the addition of β-CD lavender essential oil microcapsules (LEO-β-CD) obtained from the essential oil of the lavender flower before the final beer bottling.
The physicochemical and sensorial properties of beer samples were studied in terms of the concentration of β-CD microcapsule that gave the most pleasant taste to our consumer target group, as well as the amount of LEO-β-CDs required during dosing and safety for the consumer. The effectiveness of the preparation of microcapsules and the dosage concentrations were examined with GC-MS method using the concentration of linalool as the main essential oil component.

Keywords and phrases: β-cyclodextrin, microcapsule, linalool, sensorial properties

1. Introduction

There is a growing interest in and consumption of various beers and a growing demand for flavoured beers. For this reason, the aim of the investigation was to prepare a beer flavoured with herbs and a beer flavoured with microencapsulated essential oil to investigate which dosing method is more effective and which one provides the most beneficial sensory properties for the consumer. Based on some surveys, it can be stated that the consumers of flavoured beers are mainly women, followed by young

adults over 18 years of age. Thus, the target consumer community for this product is made up of women and young adults (*Núñez-Caraballo et al.*, 2019).

1.1. Lavender as a medicinal plant, its active substances, fields of application, and physiological effects

According to the *European Pharmacopoeia*, 11th edition (*Ph. Eur.* 11, 2022), the drug can be the essential oil produced by steam distillation from the dried flowers of lavender and the fresh flower buds and less frequently from the flowering shoots of lavender species. The pharmacopoeial-grade drug shall contain at least 13 ml/kg of essential oil, containing 25–47% of the ester expressed as linalyl acetate. The French lavender flower drug contains 0.5–3% essential oil, mainly linalyl acetate (30–60%) and linalool (20–50%). The hybrid lavender flower contains essential oil (0.9–5%), but the proportions of the components vary.

According to *Oroian et al*'s research, the ratio of essential oil components in *Lavandula angustifolia* fresh stalked flower and stalkless flower is as follows: linalool 25.3–43.0% and 25.5–42.1%, linalyl acetate 3.6–37.4% and 8.0–47.2%, and camphor 0–9.8% and 0–11.4% respectively (*Oroian et al.*, 2019).

Lavandula angustifolia has sedative, bile-secreting, and antibacterial effects, and the decoction made from the drug is used for sleep disorders, restlessness, "indigestion", flatulence, and gallstones (*Mardani et al.*, 2022; *Batiha et al.*, 2023; *Firoozeei et al.*, 2021). Lavender essential oil (LEO) is used externally in ointments or as a rubbing agent in an alcohol solution to treat rheumatic and nerve pains and reduce skin aging. The largest user is the cosmetics and perfume industry (*Prusinowska & Śmigielski*, 2014; *Henriques et al.*, 2020). It has been used recently in food production for the natural flavouring of (mainly alcoholic) beverages, ice creams, confectionery, bakery products, and chewing gum *(Da Porto et al.*, 2009).

1.2. Characterization of Blonde Ale beers

Blonde Ale beers are easy-drinking, approachable, malt-centric American craft beers, often with interesting fruity, hoppy, or malty notes. Well-balanced and clean, it is a refreshing beer without intrusive flavours. The aroma ranges from light to moderately sweet malty, with possible slight bread or caramel notes appearing. Low to moderate fruitiness may be present, which is acceptable, and low to medium intensity hop aroma; almost any hop variety may be present, although citrus, floral, fruity, and spicy notes are common. The colour can range from light yellow to dark gold, clear to bright, with little to medium white foam, and the persistence ranges from acceptable to good. The flavour shows a soft, malty sweetness to start but may optionally show light, malty flavours (e.g. bread, biscuits, toast, wheat). Caramel flavours are typically absent, but if present, pale caramel notes are typical

characteristics. Few to moderate amounts of esters are optional but acceptable; hop flavours (of any type) range from light to moderate and are not overly aggressive. There is a medium to low bitterness, but the balance leans towards malt or more malty rather than hoppy. From medium dry to malty sweet finish – the impression of sweetness is often due to the perceived bitterness being lower than the actual residual sweetness (*Gordon & Kristen*, 2022).

1.3. Dosing options for lavender in beer

Lavender has a relatively strong aroma, so the amount to be administered should be determined accordingly. Dosing can be done in several ways, first considering the plant or the essential oil made from it. Having studied the process used by several small producers, the conclusion is that the plant itself is preferred for flavouring. Dosing options also offer two possibilities: addition during the hop boiling process or fermentation process. Based on a previous thesis (*Ignácz*, 2013), dosing during boiling was found to be better based on the sensory analysis of the final product, and this method was used to produce lavender-flavoured control beer. Another possibility was explored in parallel: the dosing of essential oil mentioned above, which is introduced into the product using a carrier material that can also be used in the food industry immediately before bottling. For this purpose, β-cyclodextrin was used, a carrier with recognized and accepted properties in the food industry but not yet used by the brewing industry.

1.4. Cyclodextrin (CD) as a food carrier

Despite the importance of CDs in food, the last complete study on their use in food science was published in 2009. The article covers the characteristics of the most important industrial-grade CDs (α-CD, β-CD, and γ-CD) and their main technological properties such as solubility and ability to form inclusion complexes. It also includes the current technology for using these compounds in the food industry (*Matencio et al.*, 2020). CDs are torus-shaped oligosaccharides composed of α-(1,4)-type glucose units produced from the breakdown of starch by the enzyme cyclodextrin glucosyltransferase (CGTAse). The CD ring is an amphiphilic cone-shaped cone with a hydrophilic outer part (by hydroxyl groups) and a predominantly lipophilic cavity that may contain water (*Pereva et al.*, 2019). CDs have a promising future due to consumer demand for healthy and functional products (*Pereira et al.*, 2021).

1.5. Metabolism and toxicology of cyclodextrin

In nature, only CGTAse can convert starch into CDs, although other enzymes can help in their industrial production (*Matencio et al.*, 2020); however, different

enzymes or processes in our body can break down CDs into glucose derivatives. In the mouth (if we consume something containing CDs), salivary α-amylase can rapidly hydrolyse dextrins although rapid transport to the stomach means that the degradation rate is insignificant. Of the three natural CDs (α-, β- and γ-CD), the first two are essentially stable in the presence of α-amylase, whereas γ-CD is rapidly digested (*Matencio et al.*, 2020).

Specific pH-dependent degradation can occur in the stomach, but lower degradation is observed in the presence of complexes (*Matencio et al.*, 2020). After the stomach, in the neutral pH environment, the pancreatic amylase of the small intestine continues the hydrolysis reaction. While α- and β-CDs are mainly digested by bacteria in the large intestine (where α-CD is more rapidly degraded than β-CD), γ-CD is almost completely digested in the gastrointestinal tract. Finally, the undegraded CDs are metabolized by microbiota in the last stage of the digestive tract, where they are almost completely degraded, being used as prebiotics in their life functions (*Fenyvesi et al.,* 2016), the rest being excreted in the faeces.

Generally, the bioavailability of natural and more relevant CD derivatives is very low, making them safe for oral administration (*Matencio et al.*, 2020). As their molecular weight increases, linear dextrins and CDs are increasingly excreted in the urine. Indeed, molecules smaller than 15 KDa are almost entirely excreted in the urine with almost no modifications. More than 90% of CDs are excreted in the urine.

When used as food additives, natural CDs are classified as such (E457, E458, and E459) and are accepted as "Generally Recognized as Safe" (GRAS). The recommendation of the Joint FAO/WHO Expert Committee on Food Additives (JECFA) has set a maximum recommended level of 5 mg/kg/day for β-CD in food. On the other hand, due to their favourable toxicological profile, there is no Acceptable Daily Intake (ADI) for α and γ-CD. The European Food Safety Authority (EFSA) has confirmed the positive health effects of α-CD as dietary fibre and its suitability for reducing post-prandial glycaemic responses (*EFSA Panel on NDA*, 2012).

The dose of β-CD was reassessed in 2016 and accepted without modification at 5 mg/kg/day (*Matencio et al.*, 2020; *Fenyvesi et al.*, 2016; *EFSA ANS Panel*, 2016), with the safety of CDs being assessed and consumption levels in food being established, supporting the view that there was no need to modify the already established levels. Generally recognized as safe (GRAS) molecules, they are directly approved for use as excipients (such as natural CDs). In this regard, the US Food and Drug Administration (FDA) has published a list of inactive pharmaceutical ingredients, which includes recommended minimum and maximum consumption concentrations. A question-and-answer document on CDs and their use (*Committee for Human Medicinal Products*, 2017) provides information on their safety: for example, the consumption threshold for β-CD for oral use is 20 mg/kg/day (*Committee for Human Medicinal Products*, 2017).

2. Materials and methods

2.1. Description of the beer recipe

To produce 50 L of wort, 7.5 kg of Pale Ale malt (Weyermann), 63 g of Cascade hops (Yakima chief) with 5.7% alpha acid content, 11.5 g of Safale US-05 yeast (Lallemand), and 55 L of pre-boiled and cooled tap water were used.

2.2. Description of the wort brewing process

In order to set up the experiment, a Ziptech NaNo small-scale brewing machine was used to brew approximately 50 L of wort. The mashing was carried out using 7.5 kg of Pale Ale malt and 25 L of pre-boiled and cooled mashing water. For mashing, the following temperature programme was used: holding at 65 °C for 60 min, holding at 72 °C (1 °C/min) for 30 min, increasing to 75 °C for 5 min, and holding at 78 °C for 1 min. 30 L water was used for the crawling.

2.2.1. Preparation of lavender-flavoured beer

At the start of the hop boil, 25 g of lavender flowers and 42 g of Cascade hops with 5.7% alpha bittering acid are added. At the end of the 60-minute boil, 50 g of lavender flowers and 21 g of Cascade hops are added. The resulting wort was settled for 30 minutes and, after cooling (28 °C), it was pumped into the fermentation tank, where it was inoculated with 11.5 g Safale US-05 pre-hydrated yeast and fermented at 22 °C for seven days. After the primary fermentation, the temperature of the young beer was reduced to 4 °C, and after two days, the yeast was removed, and the maturation continued for fourteen days, after which the finished beer was bottled in 0.5 L bottles and stored in a beverage refrigerator at 4 °C.

2.2.2. Preparation of beer flavoured with β-CD lavender essential oil microcapsules (LEO-β-CD)

For the dosing of the LEO-β-CD described in section 2.2.3, again, the beer recipe described above was used, dosing the microcapsules at the end of the primary fermentation before bottling. Dosing was carried out at several concentrations, summarized in *Table 1*. After dosing the LEO-β-CD, the flavoured beers were stored at 4 °C. The quantities of LEO-β-CD added were determined considering the expected essential oil content of the beer containing lavender flowers. The recipe shows that 75 g of flowers were added to 50 L of beer. Since the essential oil was previously extracted from lavender flowers by steam distillation, it can be observed

that the essential oil content of the flowers is 2.7%, so the amount of essential oil added together with the flowers can also be determined, which was 2.02 grams. The hop flower beer contained approximately 0.0434 g of essential oil per litre. Knowing the amount of essential oil added in the production of the LEO-β-CD (3.6 mL), taking into account the density of lavender essential oil (0.885 g/cm^3), we determined the mass of essential oil added (3.186 g) and then the number of grams of essential oil contained in one gram of LEO-β-CD (0.155 g).

Based on the above data, it was estimated that 0.287 g of LEO-β-CD is required to obtain 0.0434 g/L of essential oil in beers produced with LEO-β-CD. Since the above calculation involves estimating several values, five beers produced with LEO-β-CD at different concentration levels (two lower, one similar, and one higher volatile oil concentration) were prepared using the amounts of LEO-β-CD shown in *Table 1*.

Table 1. The LEO-β-CD dosage amounts

Sample ID	**1**	**2**	**3**	**4**	**5**
LEO-β-CD concentration [g/g]			0.155		
Amounts of LEO-β-CD in beer [g/L]	0.016	0.072	0.107	0.144	0.21

2.2.3. Preparation of LEO-β-CD

For the preparation of LEO-β-CD, the recipe published by *García-Segovia et al.* (2011) was used. In the first step as per the recipe, a 30 mL, 12% essential oil-ethanol solution was prepared. For this purpose, 3.6 mL of lavender essential oil obtained with steam distillation and 26.4 mL of ethanol (96 v/v%, Sigma Aldrich) were poured and mixed using a magnetic stirrer. Next, a 10% β-CD solution was prepared using 220 ml of ethanol-water solution in a 1:2 ratio and 22 g of β-CD (Cyclolab). The ethanol-water solution contained 146.6 ml distilled water and 73.3 ml ethanol (96 v/v%, Sigma Aldrich).

β-CD was added to the ethanol-water mixture with constant stirring using a magnetic stirrer with heating. To the 10% β-CD solution, set at 55 °C, the 30 mL essential oil-ethanol solution was slowly added, drop by drop, with constant stirring. Following the addition, mixing continued for four more hours, then cooled and maintained at 4 °C for 12 hours. After settling, the microcapsules were separated from the liquid, using a vacuum filter with 0.40 µm porosity and dried at room temperature (22 °C) for 24 hours. In order to ensure a longer shelf life of the LEO-β-CD prepared, they were dried in a drying oven at 50 °C for another 24 hours and stored in an airtight glass container until use.

2.3. Sensory analysis of the beer

The sensory analysis was conducted in the Fermentation Laboratory of the Department of Food Science, Sapientia Hungarian University of Transylvania, which meets the conditions described in the Romanian SR 13355-1/1997 standard, with light-coloured furniture, white walls, and a room free of foreign odours and noises. Natural light was available during the tasting, without direct sunlight, which allowed the correct assessment of the colour of the beer. At each tasting point, a plate with bread was placed to eliminate any residual flavour, and a bowl was placed to collect the leftovers from the tasting.

A focus group carried out sensory evaluations of beer samples with 26 people (14 male and 12 female) aged 18–25 years, who were also selected as the target consumer group. The tasters evaluated our beer samples using a tasting questionnaire that was compiled to assess the external appearance, colour, smell, taste, CO_2 content, foam appearance, and durability, using a 0–5 hedonic scale (0: very bad, 5: excellent). The values obtained were weighted by a constant: external appearance (0.6), colour (0.8), smell (0.2), taste (1.4), CO_2 content (0.6), and foam stability (0.4). The acceptance test's final scores were based on the classification provided in SR 13355-1/1997, and the following categories were distinguished: excellent (20.0–18.1), good (18.0–15.1), agreeable (15.0–12.1), unsatisfactory (12.0–7.1), and bad (7.0–0.0).

Carbon dioxide impregnation was evaluated simultaneously with the evaluation of appearance and taste. The odour was assessed immediately after opening the bottle. Six beer samples were subjected to sensory analysis, which were analysed in the following order: 1st sample: 0.016 g/L LEO-β-CD; 2nd sample: 0.072 g/L LEO-β-CD; lavender-flower-flavoured; 3rd sample: 0.107 g/L LEO-β-CD; 4th sample: 0.144 g/L LEO-β-CD; 5th sample 0.21 g/L LEO-β-CD. A 2-minute break was taken between tasting the samples. Tasting participants recorded their ratings on a tasting form developed online (Sensory evaluation of lavender-flavoured beers).

2.4. Determination of the physicochemical properties of beer

The original extract, sugar, alcohol, CO_2, O_2, density, and beer turbidity were determined using an Anton Paar PBA-B (Packaged Beverage Analyzer for Beer) analyser according to EBC 9.43.2/2004. For the pH and colour measurements (EBC 9.35/2004), CO_2 was removed from the beer by shaking. Since our sample was an unfiltered beer before the colour determination, the samples were filtered with kieselguhr filter powder according to EBC 9.6/2000 and then measured with a spectrophotometer (Hach Lange DM 6000) at $\lambda = 275$ nm.

The bitterness of the beer was determined according to EBC 9.8/2004. The sample was centrifuged at 4,000 rpm for 20 min at 20 °C. 1 mL of 3 M HCl and 20 mL of isooctane were added to 10 mL of beer sample, shaken for 25 min

on an IKA shaker at 450 rpm, left in the dark for 30 min, and measured with a spectrophotometer at λ = 275 nm.

2.5. Determination of linalool content

The main component of lavender oil, linalool, was determined using the method published by *Kishimoto et al.* (2006). Hexane (3:1) was added to the beer samples and shaken at 4 °C for 24 hrs at 160 rpm. The supernatant was removed by pipette, dried with Na_2SO_4, and 1 µL was injected into the GC-MS. Lavender essential oil and LEO-β-CD were also dissolved in hexane. The GC-MS data were the following: Agilent 7890 A-5975C GC-MS, HP-5MS capillary column (30 m × 0.25 mm × 0.25 µm). Carrier gas was helium at a flow rate of 1 mL/min. MS settings were: ionization voltage (EI), 70 eV; ionization temperature, 250 °C; quadrupole temperature, 150 °C; quantitative scanning range: 35–350 u. For qualitative analysis of linalool MS NIST database was used (retention time 7.43 min). For the quantification of linalool, a calibration was performed over a concentration range of 0–2 mg/L, where the equation of calibration line was A = 33350.0 ·Clinalool + 303.1 (correlation coefficient R2 = 0.9970), obtained by linear regression.

2.6. Statistical analyses

All data were expressed as the means of the measurements ± standard deviation and were made in triplicates. The one-way analysis of variance (ANOVA) with Tukey's test was employed to evaluate the significant differences at $p < 0.05$. Statistical tests were made using XLSTAT software for the Excel 2021 version (Addinsoft, New York, NY, USA).

3. Results and discussions

3.1. Results of the sensory test and their evaluation

The test was carried out according to the Romanian standard SR 13355-1/1997, using an online questionnaire, which we designed following the questions of the standard. During the tasting, 26 participants gave their opinions on beer samples containing different concentrations of LEO-β-CD and beer samples produced with lavender flower dosage. The participants were between 18 and 25 years old, and the gender distribution was 55% men and 45% women.

From the general questions (How often do you drink beer? What characteristics do you consider when choosing a beer? Which type of beer do you prefer based on taste? Which type of beer do you prefer based on alcohol content?), it was found

that men drink alcohol more often and base their choices on taste and flavour, prefer less bitter beers, and mainly drink beers with 4-5% alcohol. Women's beer consumption was less frequent, once a month, and, like men, women chose beer based on taste and aroma. Opinions were divided on the classification by flavour, but the majority preferred flavoured beers. In terms of alcohol consumption, women prefer beers with an alcohol content of 4-5% v/v.

After the tasting, the acceptance test results (*Table 2*) showed that the beer samples produced were suitable for consumption and contained minor defects. These are the low CO_2 content and the low foam, which are mostly due to the fact that the laboratory does not yet have bottling technology to adjust the CO_2 content in the product. The low CO_2 content also negatively affects foam stability (*Figure 1*).

At the acceptance test, lavender flower beer (13.5) and a beer sample containing 0.072 g/L LEO-β-CD (13.51) achieved the highest scores. Otherwise, this result is promising, as this feedback confirms that LEO-β-CDs can be a good option for flavouring beers (*Table 2*).

Table 2. Acceptance test results

Properties	**Beer samples**					
	1	2	Lavender flower	3	4	5
	Amount of LEO-β-CD in beer, g/L					
	0.016	0.072	-	0.107	0.144	0.210
	Weighted average score values of beer samples					
External appearance	2.03 ± 0.67[a] *	2.26 ± 0.76[a]	2.42 ± 0.73[a]	2.17 ± 0.75[a]	2.22 ± 0.54[a]	1.98 ± 0.78[a]
Colour	3.20 ± 0.99[a]	3.17 ± 0.89[a]	2.03 ± 1.66[b]	2.95 ± 0.91[a]	3.14 ± 0.81[a]	3.17 ± 1.03[a]
Smell	0.72 ± 0.18[a]	0.77 ± 0.23[a]	0.78 ± 0.29[b]	0.64 ± 0.21[a]	0.75 ± 0.21[a]	0.74 ± 0.26[a]
Taste	5.28 ± 1.29[a]	5.55 ± 1.50[a]	5.12 ± 1.70[b]	5.17 ± 1,64[a]	5.22 ± 1.38[a]	4.95 ± 1.85[a]
CO_2 content	1.25 ± 0.86[a]	1.22 ± 0.64[a]	2.08 ± 0.60[b]	1.29 ± 0.76[a]	1.15 ± 0.68[a]	1.52 ± 0.92[a,b]
Foam	0.43 ± 0.39[a]	0.54 ± 0.44[a]	1.08 ± 0.39[b]	0.68 ± 0.52[a]	0.68 ± 0.52[a]	0.83 ± 0.48[c]
Sum	12.90	13.51	13.50	12.90	13.15	13.20
Category	Agreeable	Agreeable	Agreeable	Agreeable	Agreeable	Agreeable

Notes: * Results represent mean values ± standard deviation (SD), n = 26; letters *a*, *b* indicate significant differences between the beer samples at $P \leq 0.05$ level; letter *c* indicates significant differences between sample 1 and sample 5 (at $P \leq 0.05$ level).

The external appearance of lavender-flavoured beer is that of a clear, bright liquid, without suspensions or sediment, with foam and carbonation, and is appreciated by both male and female tasters. As for the colour, amber was also the highest-scoring colour for men, while it was light yellow for women. The tasters found that the beer samples had the characteristic lavender flavour but needed to be more pronounced and balanced. The characteristic lavender aroma was present in the samples, although some tasters did not detect it. As noted in the taste assessment, the aroma has the characteristic lavender scent, as confirmed by the tasters, with one glaring case of a featureless scent.

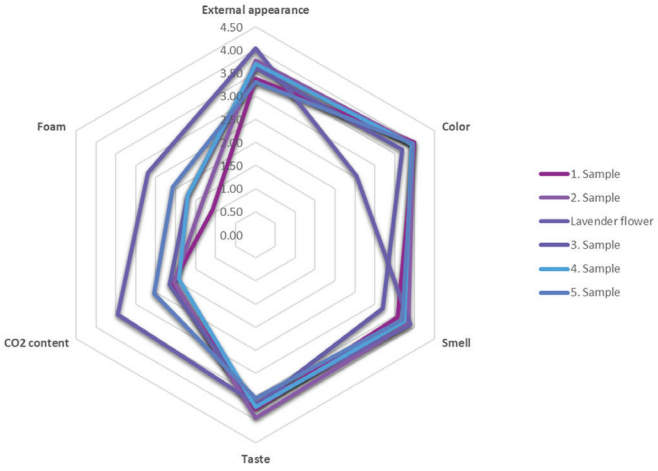

Figure 1. Results (expressed as the median) of the sensory analysis

The external appearance of the LEO-β-CD 0.072 g/L sample was acceptable, with most tasters describing it as clear and free of suspension (*Figure 2*). However, three tasters thought it contained small particles, which we attribute to its unfiltered nature. Regarding colours, most male tasters considered the sample containing 0.072 g/L β-CD amber, while the female tasters considered it more typical ruby amber. Discrepancies may be due to differences in colour perception between the sexes or individuals. The tasters did not experience any uncharacteristic beer flavour despite the β-CD used for flavouring, and the majority considered the lavender flavour to be noticeable. As with the flavour, the sample smelled of lavender, with one glaring opinion that the sample smelled of lavender was featureless, again a function of the tasters' different perceptions.

Based on the responses to the final question of the questionnaire, "Which sample number did you like the most?", women overall preferred the beer sample containing 0.072 g/L LEO-β-CD, while men who participated in the tasting preferred the lavender flower-flavoured beer sample.

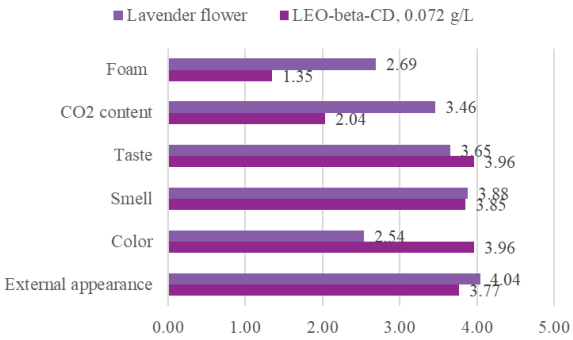

Figure 2. The acceptance test results for most liked samples

The one-way ANOVA results show (*Table 2*) statistically significant differences in the sensory scores for colour, smell, taste, CO_2 content, and foam among the six beer samples. The results of the Tukey HSD test present significant differences between the beer with lavender flowers and the beers with LEO-β-CD. Regarding the CO_2 content, the beer sample with 0.210 g/l LEO-β-CD did not show statistically significant differences from the other samples. In terms of foam, the beers with the smallest and largest amounts of LEO-β-CD microcapsules showed significant differences.

3.2. Main physicochemical properties of the base beer

Taking into account the BJCP – Beer Judge Certification Program standard, the lavender beers we produce (since the base beer is the same, and therefore the physicochemical properties are the same except for the aroma), as evidenced by the physicochemical properties defined (*Table 3*), can be classified as Blonde Ale.

Table 3. Physicochemical properties of beer samples

Properties	**Lavender beer**	**Blonde Ale**[1]
Original extract [Plato]	8.91 ± 0.02	11.00
Apparent extract [%]	1.30 ± 0.02	–
Alcohol content [% v/v]	3.96 ± 0.01	3.80-5.50
Density [g/cm^3]	1.00345 ± 0.0001	-
CO_2 [g/l]	1.368 ± 0.002	-
O_2 [mg/l]	3.20 ± 0.01	-
Calorie [kJ/100ml]	132.23	-
pH	5.13 ± 0.01	-
Colour [EBC]	13.90 ± 0.02	5.90–11.80
Bitterness [IBU]	24.60 ± 0.10	15.00–28.00

Note: [1] BJCP – Beer Judge Certification Program.

3.3. Results of the linalool content of beer samples

The GC-MS measurement showed that the linalool content of lavender flower essential oil used for LEO-β-CD production was 33%, which fit within the limits found in the literature (25.3–43.0% – *Kara & Baydar*, 2012), and the linalool content of LEO-β-CD was 45 mg/g microcapsule.

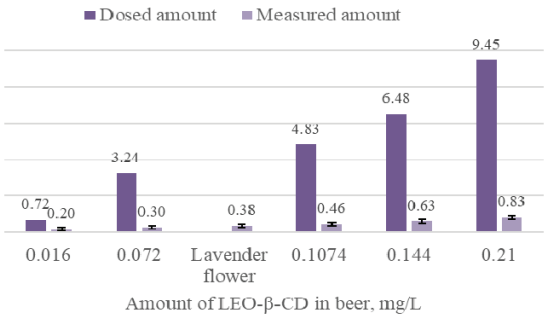

Figure 3. Linalool concentration calculated based on the dosed (45 mg linalool/g LEO-β-CD microcapsule) and measured amount in different beer samples

The measured values of linalool concentration are around 10% of the dosed amount, and increasing the dosed amount does not increase the measured value, so further experiments are needed to confirm this by examining whether the amount of solubles does not increase with higher amounts of LEO-β-CD.

The following graph shows the amount of linalool already present in the samples, expressed as a percentage.

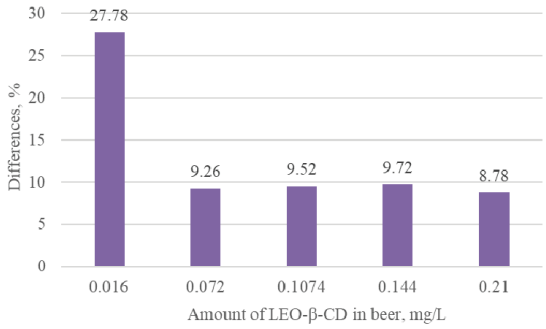

Figure 4. Percentage differences between the calculated and the determined linalool concentration

Concerning the β-CD microcapsule dosing of the beer samples, the recovery efficiency of linalool was around 9% on average. The lowest concentration of LEO-β-CD beer (0.016 g/L) has a very high recovery of 27.7%, which was attributed to the sample preparation method, but further measurements are needed to confirm this.

3.4. Safety aspects of the consumption of CD-microcapsule-flavoured beers

The safe consumption limit for β-CDs is 5 mg/kg/day (EFSA, 2016) or 20 mg/kg/day for oral use (EMA, 2017). The daily consumption of β-CDs can vary between 325 mg/day and 1,300 mg/day for a person with an average body weight of 65 kg. Our beer samples with LEO-β-CD contained between 0.013 g β-CD/L and 0.178 g β-CD/L of β-CD, so we can state that none of the samples exceeded the safe daily intake of β-CD. In the light of this, it was calculated that 1.8 L of beer containing the highest amount of LEO-β-CD (0.178 g β-CD/L) can be safely consumed in one day.

4. Conclusions

Based on our results, both dosing methods can be used for flavouring. However, in the case of beers flavoured with cyclodextrin microcapsules, the influencing factor is the amount of microcapsules added. Acceptability tests show that consumers preferred lavender-flavoured beer to the same extent as beer containing 0.072 g/l LEO-β-CD.

Our beers, containing lavender flowers and LEO-β-CD, are suitable for consumption based on their organoleptic and physicochemical properties. The sensory tests have shown that the beer samples containing lavender flowers and 0.072 g/L LEO-β-CD best meet the requirements of SR 13355-1/1997.

None of our beer samples exceeds the safe daily intake of β-CD, and at least nine (0.5 L) bottles of beer (4.5 L) containing the most preferred beer sample with LEO-β-CD (0.072 g β-CD/L) could be safely consumed per day.

Further research is needed to develop recipes for beer samples containing 0.072 g/L of LEO-β-CD, focusing on balancing the hop and lavender flavours.

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