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Physical and Chemical Properties, Flavour and Organoleptic Characteristics of Walnut Purple Rice Yoghurt

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Abstract: In recent years, green and healthy foods have attracted much attention. Plant-based foods have become a new trend to replace animal-derived foods. In this study, we used walnut and purple rice as the primary raw materials to produce yogurt. The process included boiling, mixing, grinding, inoculation, fermentation, and sterilization. We then analyzed the similarities and differences between the resulting walnut purple rice yogurt and unfermented walnut purple rice milk, as well as dairy-based yogurt, in terms of physical chemistry, flavor, and sensory characteristics. We also examined the similarities and differences between fermented walnut purple rice milk and conventional warm yogurt. The study results revealed that walnut purple rice yogurt exhibited greater viscosity than walnut purple rice milk and conventional dairy-based yogurt. Additionally, the former displayed enhanced stability and recovery ability. Notably, distinguishable differences were observed between the three types of yogurts in terms of the presence of unknown volatiles and the umami signal as indicated by electronic nose/tongue and GC-IMS analyses. The umami flavour of walnut purple rice yogurt surpasses that of traditional dairy-based yogurt, whilst its salty taste is lower than that of walnut purple rice milk. Despite possessing a weaker aroma than dairy-based yogurt, it is more potent than walnut purple rice milk. Additionally, its relative abundance of olefins, ketones, and alcohols enhances its unique flavour profile, surpassing both other options. Based on sensory analysis, it can be deduced that walnut purple rice yogurt has the highest overall acceptance rate.

Keywords: walnut purple rice yogurt; common normal temperature yogurt; rheology; flavor; sensory

1. Introduction

As the global population expands, there will be a significant increase in the demand for food, specifically protein-rich foods [21]. Milk is a nutritious source of protein, calcium and vitamin D. However, high levels of saturated fat in milk can adversely affect human health [38]. Furthermore, the escalation of global warming, the increase in chronic ailments, and the emergence of populations with lactose intolerance or malabsorption, milk protein allergies, vegetarianism, and light diets have all played a role in the surge of milk alternatives [30]. Plant-based proteins offer numerous benefits over animal-based sources and are increasingly becoming a groundbreaking and rapidly expanding aspect of various food sectors [4]. Traditional yoghurt is commonly produced using cow's milk, whereas plant-based yoghurt is typically derived from protein-rich plants. Plant-based yoghurt is frequently utilised as a milk alternative owing to its biologically active constituents and the

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advantages it has for human health [39]. Overall, plants and animals have complementary and symbiotic roles in healthy and sustainable food systems [11].

The walnut, belonging to the Juglandaceae family, has been acknowledged as a nutrient-dense food in various parts of the globe [43]. Walnut kernels contain a high concentration of protein, fat, vitamins, minerals, fibre, and polyphenolic compounds [35]. Containing unsaturated fats and polyphenols, it offers myriad health benefits, including reducing the risk of cardiovascular and coronary heart disease, treating type II diabetes, preventing and treating specific cancers, and alleviating symptoms associated with aging and neurological diseases [32]. China possesses abundant walnut resources and boasts a lengthy history of planting. It holds the title of the world's major producer of walnuts [4]. Purple rice is a glutinous rice variety that is highly valued as a coloured rice germplasm resource in China due to its long history of cultivation. It belongs to the glutinous rice family. Purple rice is a glutinous rice variety that is highly valued as a coloured rice germplasm resource in China due to its long history of cultivation [13]. Research has demonstrated that the nutritional elements of coloured rice, including protein, amino acids, and minerals, surpass those of regular white rice [37]. Purple rice contains flavonoids and phenolics, both bioactive compounds [12]. Anthocyanins, the major components of pigmented rice, exhibit a range of biological activities including free radical scavenging, antioxidation, anti-tumour, anti-atherosclerosis, hypoglycaemic and anti-allergic effects. These activities are vital for preventing diseases and promoting health, making pigmented rice an attractive candidate for food and pharmaceutical applications [22]. Walnuts and purple rice are high-quality plant-based foods with significant nutritional and medicinal benefits. They contain numerous bioactive substances, including phenolic compounds, unsaturated fatty acids, and anthocyanins, that are beneficial to human health. The combination of walnuts and purple rice not only enriches nutrition but also enhances the unique flavour, making it an excellent choice for dairy-free milk alternatives.

Plant-based yogurt is fermented by lactic acid bacteria using plant protein as the raw material. Lactic acid bacteria can acidify the environment and break down plant compounds with enzymes, which can change the structure and sensory characteristics of the product and improve the bioavailability of the plant matrix components [28]. Fermentation can also improve food safety, extend shelf life, and add nutritional and sensory value [19]. Plant-based yoghurt has unique nutritional benefits and excellent product safety. It has great potential for market development as an alternative or supplement to animal milk. An increasing number of studies have shown that mild fermentation can improve the taste and flavour of plant-based products. This study aimed to investigate the effect of Lactobacillus plantarum fermentation on the flavour and nutritional value of walnut purple rice milk and to further analyse the difference between walnut purple rice yoghurt and ordinary room temperature yoghurt. This experiment will provide a theoretical basis for the development of walnut purple rice yoghurt.

2. Materials and Methods

2.1. Raw Materials

Dehulled walnut kernel, purple rice, fresh cow's milk, sucrose, carboxymethyl cellulose CMC, modified starch, rapeseed oil, Lactobacillus plantarum(GDMCC accession numbers GDMCC1.2685)

2.2. Strains and growth conditions

First, activated Lactobacillus plantarum was inoculated in 100mL MRS broth medium, inoculated in 2%(v/v) inoculum, and incubated at 37°C for 16 hours to obtain starter cultures for fermentation. Afterwards, the concentration of Lactobacillus plantarum was adjusted to 1 × 108 CFU/mL, and then centrifuged at 5000rpm for 5 minutes at 4°C ,washed three times with sterile saline, and recovered as a seed solution for fermentation.

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2.3. Sample Preparation

Slightly adjusted according to the existing formula and process in the laboratory.

Walnut purple rice fermented milk :After washing, the purple rice was soaked in deionized water for 2 hours. After soaking, the purple rice was heated at 95°C for 30min, and the same amount of peeled walnut kernel was added, and then grind with deionized water (80°C) in a ratio of 1:3. Walnut purple rice milk was finely ground in a colloid mill for 15 min. Weigh 8.5% sucrose, 0.3% CMC and 0.5% rapeseed oil and add them to walnut purple rice milk. The mixture was stirred with a stirrer, heated at 95°C for 1.5h, cooled to 34°C, and inoculated with 1%(v/v) culture. The walnut purple rice fermented milk (H1) was obtained by fermentation at 34°C for 14 h and sterilization at 85°C for 30min, and stored at room temperature.

Walnut purple rice milk:Under the same process and formula, the unfermented walnut purple rice milk (H2) was used as the control group and stored at room temperature.

Ordinary room temperature yogurt :Fresh milk was preheated to 45°C, added with 8.5% sucrose, 2% modified starch and 0.6% compound thickening emulsifier HBT-A8570, and heated at 65°C for 15min. Then, the emulsion was homogenized at 25MPa for 5min using a high-pressure homogenizer (APV-2000, SPX FLOW Inc., Charlotte, NC, USA). Then the emulsion was pasteurized at 85°C for 2min in a stirred water bath (Lochner mashing device, LP electronic, Berching, Germany). The sterilized mixed milk was cooled to 37°C and inoculated with 0.5‰ commercial starter. The fermentation was carried out at 42°C for 6h, and then sterilized at 85°C for 30min to obtain dairy based yogurt (C1).Storage at room temperature, Each treatment is in triplicate.

2.4. Physicochemical analysis

Titration acidity (TAA) :10g samples were weighed in a conical flask, 10mL of distilled water was added, shaken, 2~3 drops of 0.5% phenolphthalein indicator were added, and titrated with 0.1mol/L NaOH solution to pink and maintained for half a minute. The consumption of NaOH solution was recorded, and the titratable acidity of each group of yogurt samples was calculated. Repeat three times to take the average as the final titration acidity.

pH: The fermented milk was restored to room temperature and stirred evenly with a glass rod. The pH value of the fermented milk was measured by a pH meter, and the sample was measured in triplicate.

The samples were ana-lyzed for chemical composition (protein, fat, total solids, and ash)using standard AOAC procedures [6].

2.6. Rheological Analyses

2.6.1. Relationship between strain and time

Under the condition of 25°C, 10 Pa shear stress was applied to the sample, and the change of strain with time was recorded for 5min, and the recovery after removing the stress was measured.

2.6.2. Relationship between apparent viscosity and shear rate

The samples were poured between the rheometer plates and placed at room temperature for 15min. The test is carried out with CC25 DIN fixture, and the test distance is adjusted to 500μm. The shear rate was increased from 0.01 S-1 to 1000 S-1, and 50 points were taken for data detection. The shear rate was used as the abscissa, and the apparent viscosity was used as the ordinate to draw the curve.

2.6.3. Determination of dynamic rheological properties

The detection temperature was set at 25°C, the shear strain was 0.5%, the frequency was increased from 0.1 to 10Hz, and the time was 10min. The changes of elastic modulus (G') and viscous modulus (G'') of the two starch pastes with the increase of frequency were measured.

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2.6.4. Relationship between shear rate and shear stress (thixotropic loop area)

The samples were poured between the rheometer plates and allowed to stand at room temperature for 15min. The test is carried out with CC25 DIN fixture, and the test distance is adjusted to 500μm. The shear rate increased from 0.01 S-1 to 1000 S-1, from which 50 points were taken for data detection, and then decreased from 1000 S-1 to 0.01 S-1 for data detection. Taking the shear rate as the abscissa and the shear stress as the ordinate, the curve is drawn. According to the spindle ring enclosed in the diagram, the area of the figure is calculated, that is, the area of the thixotropic ring.

2.7. Electronic nose and electronic tongue determination

Electronic nose : 10g of the sample was weighed and placed in a 100mL beaker, sealed with a double-layer preservative film, allowed to stand at room temperature for 30min, and then tested on the machine. Each group of samples was measured three times in parallel. When the sample is measured, the injection needle is directly inserted into the headspace bottle containing the sample for determination. Determination conditions : sampling time was 1 second/group ; the self-cleaning time of the sensor is 80 seconds. The sensor zeroing time is 5 seconds ; the sample preparation time is 5 seconds ; the injection flow rate was 400ml/min. The analysis sampling time is 80 seconds.

Electronic tongue : 100g of sample was weighed, 100mL of pure water was added, and the water was fully stirred to make the water and yogurt sample fully and evenly mixed. The sample was centrifuged at 3000rpm for 10min, and the supernatant was taken for testing. The samples in each group were determined at room temperature for 3 times in parallel.

2.8. Volatile flavor compounds analysis by HS-GC-IMS

5g of yogurt sample was placed in a 20mL headspace bottle, incubated at 40°C for 15min, and then the top gas was automatically inhaled by an injection needle at 85°C. Nitrogen (99.999%) was used as carrier gas and drift gas. The flow rate parameter was 2mL/min and maintained for 2min. The linearity was increased to 150mL/min within 18min and maintained for 20min. The analysis time was 20min. The IMS temperature was 45°C and the column temperature was 60°C.

2.9. Sensory Analysis

Sensory analysis of the samples was performed by a group of experienced sensory testers (n = 12, age range 20-59) recruited from the School of Food Science and Technology, Yunnan Agricultural University. The team members were asked to rate the intensity at a level of 0 to 10 of the following descriptors : smell, taste and taste. Team members were also asked to rate the overall acceptability of the samples at a level of 0 to 10. The questionnaire includes the definition of descriptors for sensory testing, which can be found in Table 1 Sensory analysis is carried out in the sensory analysis room and in duplicate within two days.

Table 1. Sensory word definition and reference sample.

QDA	Description	Definition
Flavor	Green grass flavor	Odors associated with newly cut grass
	Nuts flavor	Odors associated with nuts
	Cereal flavor	Odors associated with cereals
	Fermented flavor	Acid substances produced by fermentation of dairy products, occasionally sour smell.
Taste	Sweetness	Taste related to sucrose
	Sour taste	Taste associated with citric acid
	Bitter taste	Taste associated with caffeine

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	Salty taste	Taste related to sodium chloride
	Astringent taste	The sample is placed on the tongue to make the tongue feel convergent.
	Dispersion	The extent of the sample in the mouth without chewing.
Taste	Thickness	The force, fluidity, and thinness of the tongue to suck the sample in the spoon into the mouth or expand the sample.
	Adhesion	Mechanical texture characteristics related to the force required to move the adhesive on the object, sticky.
	Granular feeling	The number and size of particles can be perceived in the sample.
Overall	Acceptance	Acceptance of the sample

2.10. Data Analysis

SPSS version 23 (IBM SPSS Inc., Chicago, IL, USA) was used to conduct one-way analysis of variance (ANOVA) with Tukey 's test at a 95% confidence level.Values are expressed as the mean ± standard deviation. PCA and PLS-DA were performed using OriginPro 8.6 software (OriginLab Corporation, Northampton, MA, USA) and SIMCA 14.1 software (Biometric Software Developer Umetrics, Umeå, Sweden), respectively.

3. Results and Discussion

3.1. Physicochemical analysis

Table 2 shows the comparison of the physical and chemical components of the three samples. The results showed that the contents of protein, fat and solids in walnut purple rice fermented milk were higher than those in ordinary milk based yoghurt ($p < 0.05$). Acidity is a very important indicator of yoghurt fermentation. Acidity 4.5 is usually used as the end point of fermentation in yoghurt production [3]. The pH of unfermented H2 was 6.38 and was reduced to 4.45 (H1) after incubation with a mixture of Lactobacillus plantarum fermentation broth at 34°C for 14 hours, confirming the effectiveness of the fermentation process. Acidity and pH were mainly influenced by lactic acid produced by the growth of lactic acid bacteria [17]. The pH of dairy yoghurt is usually around 4.5 and the pH range in the dairy and non-dairy samples is 3.4 and 4.4. The total titratable acidity showed significant differences between dairy and plant samples. Compared to the plant yoghurt, the TTA of the dairy yoghurt was significantly higher and the difference between the two was about 30. Because walnut and purple rice contain a large amount of fibre and other substances, the total solids content of walnut-purple rice yoghurt is much higher than that of ordinary dairy yoghurt. Walnut Purple Rice Yoghurt with high fibre content enhances the effects of promoting intestinal peristalsis and improving digestive function.

Table 2. Physicochemical analysis of the different samples.

	H1	**H2**	**C1**
Protein(%)	3.02±0.04a	2.99±0.04b	2.86±0.02c
Fat(%)	6.67±0.08a	6.02±0.07b	2.93±0.04c
Titration acidity	48.93±1.70b	4.07±0.68c	78.72±1.41a
pH	4.45±0.01c	6.38±0.06a	4.54±0.03b
Total solids(%)	29.09±0.03a	28.74±0.04b	20.55±0.03c

Pour: H1(Walnut purple rice yogurt), H2:(unfermented walnut purple rice milk), C1:(dairy based yogurt);Values are expressed as the mean ± standard deviation,Different lower case letters indicate significant differences between groups for the same indicator ($P < 0.05$); the same as below.

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3.2. Rheological Measurements

Rheological properties can indicate changes in viscosity as the shear rate varies. Viscosity is a measure of a liquid's physical characteristics that resist flow under shearing, which can help to elucidate the attributes of dairy products [24]. The Newtonian fluid is an ideal fluid whose viscosity remains unaffected by the shear rate. On the contrary, the non-Newtonian fluids do not exhibit this ideal flow property [5]. Figure 1a,b display the relationship between viscosity, shear rate, and shear time for all sample groups. The results indicate that increasing shear rate and time led to a rapid decrease in apparent viscosity for each group before stabilising gradually. This observation revealed the characteristics of high pseudoplasticity and shear-thinning phenomenon of non-Newtonian fluid [1]. When the rate of shear is low, the damage level to the gel network structure inside the sample is minimal. Subsequently, as time and the rate of shear increase, the damage level to the gel network structure inside the sample increases until it is completely destroyed. Consequently, electrostatic interaction and hydrophobic interaction weaken, ultimately resulting in the phenomenon of shear thinning [9]. The viscosity of normal temperature yoghurt is lower than that of walnut purple rice yoghurt, suggesting that despite the addition of stabiliser and modified starch to normal temperature yoghurt, the higher total solids content of walnut purple rice yoghurt results in more binding force between the particles, leading to a higher viscosity. Additionally, fermentation promotes the precipitation of polysaccharides and other substances. The compacted polysaccharides are capable of modifying the structural network via non-covalent interactions and of incentivising thickening behaviour. This leads to an increase in the viscosity of the sample system and a decrease in its fluidity [10]. The shear stress aligning with the shear time of the three groups of walnut purple rice yoghurt samples showed higher values than those of the normal temperature yoghurt samples, indicating higher overall viscosity of the former. The shear stress and shear rate relationship curve of various samples can be seen in Figure 1c. The Walnut Purple Rice Yoghurt yields somewhat higher shear stress than the regular dairy yoghurt and unfermented Walnut Purple Rice Yoghurt.When an external force is exerted, there is a significant alteration in the structural composition of Walnut Purple Rice Yoghurt. Meanwhile, the thixotropic ring area of this yoghurt is smaller as compared to the other two groups, which suggests that its structural composition remains largely unaffected upon external force application. After the changes in the organizational state, the energy needed to revert to the initial state is notably lower in the walnut purple rice yogurt group compared to the other two groups. This suggests that this group is comparatively more stable and exhibits a more robust recovery ability. Prior research has demonstrated that yogurt with fish gelatin produces higher shear stress than control yogurt lacking gelatin, resulting in a stronger gel structure [20].

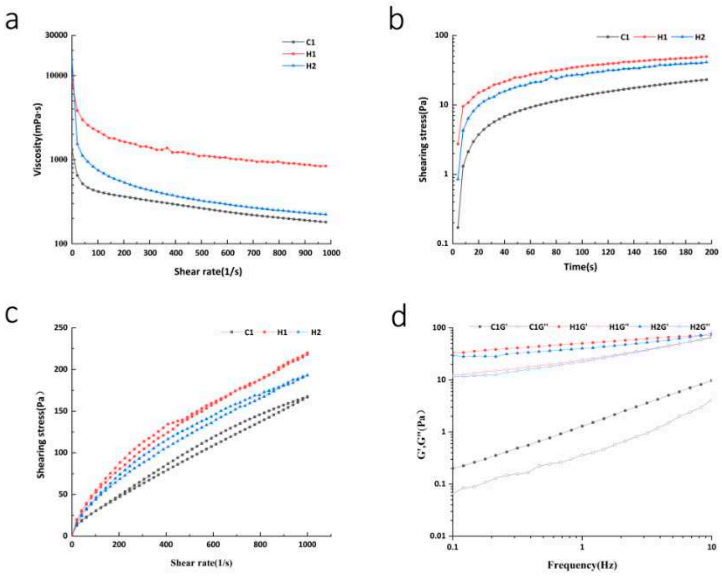

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Figure 1. (a) Relationship between viscosity and shear rate ; (b) Relationship curve between time and shear stress; (c) The relationship curve between shear rate and shear stress; (d) Viscoelastic characteristic curve.

The viscoelastic features of yoghurt specimens can be analysed further by conducting dynamic oscillation trials. Figure 1d demonstrates that upon increasing the frequency, the G' and G'' values of yoghurt samples increased. Furthermore, for the same type of samples, G' was found to be greater than G''. This indicates that the specimens exhibit a certain stiffness (elastic behaviour), presenting a semi-solid weak gel structure, in line with the characteristics of yoghurt [31]. The G' and G'' measurements of walnut purple rice yoghurt were higher than those of ordinary room temperature yoghurt, implying that walnut purple rice yoghurt exhibit more solid behaviour than ordinary room temperature yoghurt. The starch particle structure in walnut purple rice yoghurt was chain-like and dispersed, with a strong interaction between particles (gel formation). Consequently, the gel of walnut purple rice yoghurt is softer than that of ordinary room temperature yoghurt [18]. Additionally, the storage modulus (G') and loss modulus (G'') of the unpasteurised walnut purple rice milk were lower compared to those of the stirred walnut purple rice yoghurt, demonstrating that the structure of the latter was denser. This further supports the notion that fermentation enhances the gelling properties and robustness of the walnut purple rice yoghurt [7].

3.3. Analysis of electronic tongue and electronic nose

3.3.1. Electronic tongue analysis

Note : The "tasteless point" is the output of the reference solution, which comprises KCL and tartaric acid. The sour taste tasteless point is -13, the salty taste tasteless point is -6, and the tasteless points of other indicators are 0. Consequently, when the sample's taste value is lower than the "tasteless point," the sample is considered to have no taste, and vice versa. The table's richness represents the aftertaste of the umami, indicating the persistence of the umami in the sample, called the umami persistence. The bitterness aftertaste reveals the degree of bitterness residue, and the astringent aftertaste indicates the degree of astringency residue.

The Electronic Tongue is a bionic taste analysis device capable of objectively measuring food taste changes through alterations in the lipid membrane potential of an artificial taste sensor array [14]. The flavour radar images for various samples were acquired by extracting the response values from each sensor, as illustrated in Figure 2. Figure 2a shows that the acidity values for the three samples are not significant. Their acidity is lower than or proximate to the taste threshold. Additionally, other taste indicators are present above the taste threshold, substantiating their effectiveness as taste markers. H2 has lower astringency, bitterness aftertaste, and astringency aftertaste than the taste threshold. Acidity and sweetness are significant indicators of flavour across the samples. Differences between the samples' acidity and sweetness are also readily apparent. The study clearly demonstrates the distinct sweetness attributes of all three samples. The data indicate that ordinary room temperature yoghurt possesses the strongest sour taste, approaching the threshold of tastelessness while the walnut purple rice yoghurt exhibits considerably less sourness compared to the plain yoghurt (a numerical difference of several units on a standard scale).The sour taste of unfermented walnut purple rice yoghurt is significantly lower than that of room-temperature yoghurt. Additionally, the sweetness of walnut purple rice milk is greater than that of normal dairy-based yoghurt, with walnut purple rice yoghurt exhibiting the least amount of sweetness. The study results revealed higher bitterness, bitter aftertaste, astringency and astringency aftertaste scores in yoghurts as compared to odourless points, except for walnut purple rice milk. Additionally, technical term abbreviations are explained upon their first usage. It is important to note that the terms 'bitterness' and 'astringency' do not imply that the dairy products tasted extremely bitter. Instead, these terms reflect the richness, milk flavour and strong sense of taste in these products. For walnut-purple rice yoghurt, it is essential to consider the bitter taste in the results as walnut contains polyphenols and other compounds. It is crucial to account for the sensory situation of the raw material itself.The bitterness and astringency of the other two samples are lower than those of ordinary yogurt at room temperature. In particular, there is a notable difference in bitterness between the three samples, while the difference in astringency and aftertaste is minor. Richness refers to the aftertaste of umami, reflecting the persistence of umami, which can also be known as umami persistence. The study's findings reveal that the sample's richness is greater than its umami. This is because in calcium-rich samples such as yoghurt and cheese, the output of umami is inhibited by calcium, while its effect on aftertaste is unaffected.The instrument test output indicates a higher aftertaste value than the umami value, which aligns with the senses being unaware of the umami taste while perceiving the richness. The umami test revealed significant differences in umami, richness, and saltiness between the three yoghurts, with the dairy yoghurt having the lowest umami and richness and the highest saltiness. The umami of the walnut-infused purple rice yoghurt sample H1 is superior to that of C1, albeit less rich, and with the lowest saltiness score. Sample H2 possesses the highest umami and richness, with a substantial saltiness score as well.

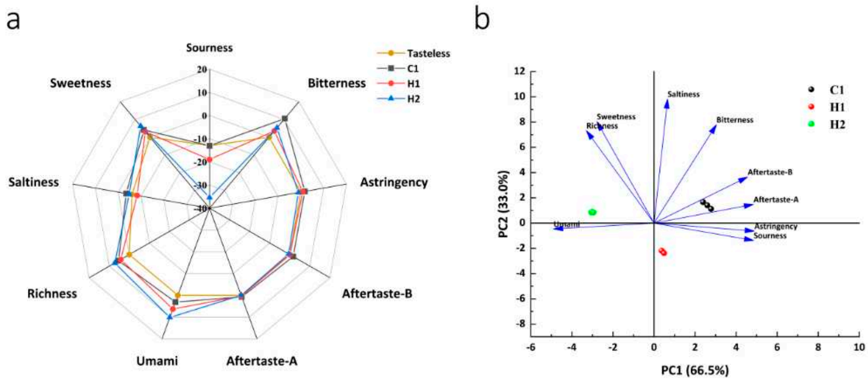

Figure 2. Radar (a) and PCA (b) of electronic tongue of different samples.

Taste is a complex sensation perceived by the taste buds, making it narrow-minded to assess a specific taste in isolation. As such, we conducted PCA analysis on the taste data from the three samples (refer to Figure 2). PCA, a widely used statistical analysis method, was employed [42],The first and second principal components were used as the horizontal and vertical axes, respectively, with variance contribution rates of 70.2% and 29.8%, respectively. Following analysis of all taste indices in this test, cluster analysis was performed on the three yoghurt samples. The graph clearly indicates that the three samples display distinct taste variations and unique characteristics. There are significant variances in the first and second principal components of the three samples. Figure 2b depicts that the first principal component is mainly impacted by sourness, bitterness and umami, while the second principal component is influenced by saltiness, sweetness, richness and bitterness. The variations in flavour profiles of the three yoghurt samples are largely discernible through these aspects.

3.3.2. Electronic nose analysis

The electronic nose and electronic tongue work in a similar way [29],The aforementioned bionic olfactory analysis technology is capable of performing a comprehensive evaluation of volatile odour information within samples in a rapid, sensitive, and non-destructive manner [36]. The radar chart, which displays the average of the results from three tests conducted on the 10 sensors of the electronic nose at 70 seconds, is illustrated in Figure 1a. Figure 3 reveals that the electronic nose has a substantial response to the three sample groups.Among them, the scent of standard yoghurt at room temperature is powerful, with the electronic nose exhibiting a significant response. Additionally, standard yoghurt, when sealed and left stationary, accumulates a relatively high concentration of headspace. In contrast, the aroma of fermented milk made from walnut purple rice is faint, and the concentration of enrichment during standing is minimal.The radar map indicates that sensors No.2, No.6, No.7,

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No.8, and No.9 respond to the three samples. Sensor No.2 is sensitive to small molecule nitrogen oxide gases, No.6 is sensitive to short-chain alkanes such as methane, No.7 is sensitive to inorganic sulphur gases, No.8 is sensitive to alcohol ether aldehyde ketones, and No.9 is sensitive to organic sulphur gases. Nonetheless, considerable variations are present in the response values of these sensors. Throughout the testing process, the C1 sensors for normal temperature yoghurt showed the highest response values, with particular sensitivity found in sensors 2 (to small molecule nitrogen oxide gas), 7 (to inorganic sulfur gas) and 9 (to organic sulfur gas). Additionally, the volatile odour of fermented milk made from walnut purple rice was markedly lower than that produced by C1; the response value from sensor H2 was especially lower than that of C1. The order of the three smells is C1 > H1 > H2. H1's response value was significantly greater than that of H2, even as most raw materials were identical. The key factor at play was lactobacillus fermentation, which stimulated the release of related compounds and led to the improvement in response value [41].

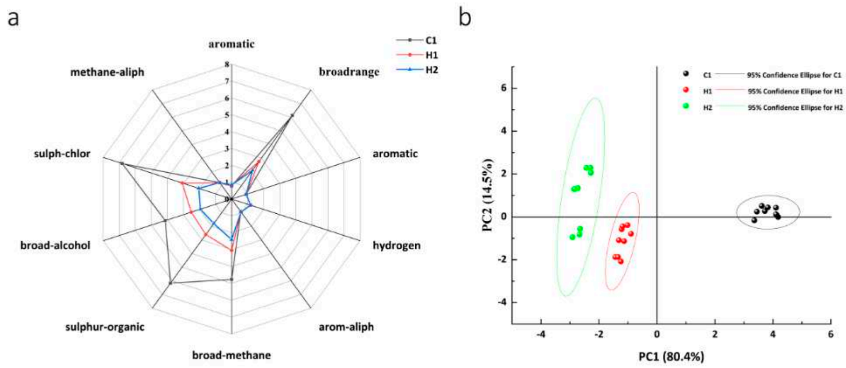

Figure 3. Radar (a) and PCA (b) of electronic noses of different samples.

In principal component analysis (PCA), the combined contribution rate of the first and second principal components amounts to almost 95%, effectively encompassing the majority of the sample's original information. Notably, the contribution rate of the first principal component is 80.4%, while that of the second is 14.5%. The scatter plots of the samples from the three groups clustered together, demonstrating good repeatability within each group and high similarity of the sample data. Furthermore, there was a clear distinction between the groups, as revealed by the mutual clustering. The electronic nose effectively discerned differences between the three samples. The normal room temperature yoghurt exhibited the largest discrepancy in odour compared to the other two samples, as indicated by the sensor readings and depicted in the figure. Furthermore, the yoghurt was significantly distinct from the other two. On the X and Y axis, the fermented milk of H2 walnut purple rice was located at a lower position, which suggests that its volatile odour is comparatively weaker than the other two samples.

3.4. GC-IMS analysis of volatile flavor compounds

3.4.1. Analysis of volatile flavor components spectrum

The fingerprint spectrum is reconstructed from all the peaks in the GC-IMS spectrum using the Gallery Plot plug-in and the characteristic peak area is identified [2]. Figure 4 displays the VOC fingerprints of various samples. The horizontal axis presents the selected characteristic identification peak, and each column corresponds to a VOC. The vertical axis denotes the sample identification numbers, and each row represents a sample. The background is coloured black and blue, whereas the red colour indicates higher content and the lighter colour shows lower content [16]. The table displays the variation of VOC content across different samples. Each row represents the type of VOC contained in the sample while each column shows the content variation. To aid observation and comparison, substances with similar variation rules are grouped and compared. These groups are then divided into four regions - A, B, C and D. Figure 4 presents a fingerprint of the volatile characteristic substances from the three samples for easy observation of their variation.The A and D areas of the map exhibit the distinctive volatile components of walnut purple rice yoghurt H1. The main characteristics include acrylonitrile, 2-butanol, 2,2,4,6,6-pentamethylheptane-M, 2-acetyl-3-methylpyrazine, and 3-heptanol. The B area (shown in the diagram) indicates the distinct volatile compounds of H2, such as ethyl trans-2-hexenoate, propionaldehyde, 1-propanol, ethyl pentanoate, (E)-2-octenal, and isopentyl formate. The substances depicted in the diagram represent the specific volatiles of H2, with a concentration significantly higher than in other samples, mostly esters. The substances depicted in the diagram represent the specific volatiles of H2, with a concentration significantly higher than in other samples, mostly esters. It is worth noting that technical abbreviations are explained on their first use to ensure comprehension. The C region in the diagram includes volatile substances such as (-)-perillaldehyde, 2-phenylethyl acetate, methylpyrazine, 1-propanol, and ethyl benzoate, which are characteristic of H2. The characteristic volatile substances of C1 are represented by the peak colours which are significantly darker in the three samples. This indicates a higher content of these substances than in the other samples. Objective evaluation is prioritized, and technical term abbreviations are explained.

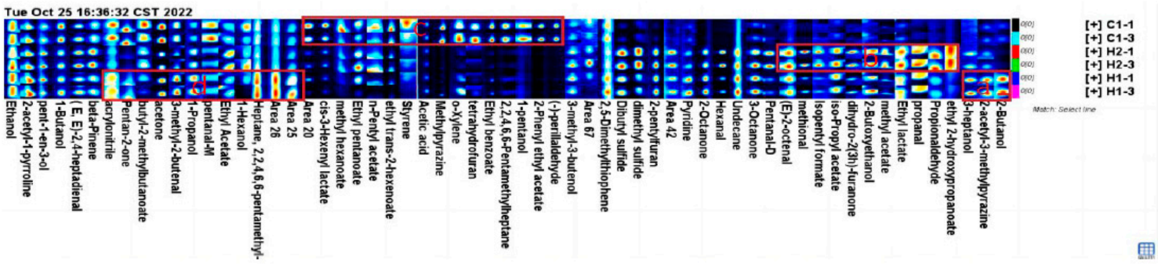

Figure 4. Gallery Plot of sample. Note: The graph displays all the signal peaks chosen in a sample, with each column showing the signal peaks of a specific volatile organic compound in diverse samples. Certain substances have the abbreviations -M and -D, referring to Monomer and Dimer of the same element. The numerical value represents an unidentified peak.

3.4.2. Analysis of relative content of volatile substances

During the examination of GC-IMS outcomes, there is a positive correlation between the characteristic peak intensity of an aroma substance and its content in the sample. The study identified a total of 55 chemical compounds, consisting of 5 ketones, 10 alcohols, 9 aldehydes, 13 esters, 2 acids, 2 ethers, 8 olefins and 8 heterocyclic compounds. Among these compounds, ketones, alcohols, esters, and aldehydes were the primary volatile compounds of both samples. By conducting a relative content analysis, we have identified 21 significant flavour compounds (with relative content greater than 1%) present in walnut and purple rice yoghurt. These compounds enhance the overall flavour of the product.

Table 3. Results of volatile flavor components of samples.

Compound	Relative retention index	Relative amount			The odor description
		H1	H2	C1	
			Aldehydes		
(-)-perillaldehyde	1153.4	0.58±0.01%	0.49±0.03%	1.39±0.04%	Perilla incense
(E, E)-2,4-heptadienal	1009.5	0.56±0.06%	0.53±0.031%	0.33±0.03%	Melon and fruit fragrance
3-methyl-2-butenal	762.6	2.94±0.05%	1.52±0.06%	1.83±0.03%	Almond, Roasted
Hexanal	1071.2	1.24±0.01%	2.51±0.08%	0.40±0.03%	Apple, Fat, Fresh, Green, Oil，
Pentanal-D	978.2	0.21±0.00%	0.41±0.01%	0.17±0.00%	Grass smell
(E)-2-octenal	1070.2	0.61±0.01%	0.70±0.01%	0.18±0.01%	Dandelion, Fat, Fruit, Grass, Spice
methional	906.1	0.82±0.01%	1.53±0.06%	0.38±0.02%	Savory
Propionaldehyde	819	0.44±0.01%	0.59±0.02%	0.11±0.00%	Floral, Pungent, Solvent

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pentanal-M	977	0.30±0.01%	0.25±0.01%	0.39±0.02%	Grass smell
			Ketone		
Pentan-2-one	980	0.19±0.03%	0.15±0.01%	0.15±0.06%	Flowers, fruit fragrance
dihydro-2(3h)-furanone	908.7	0.51±0.02%	0.82±0.03%	0.26±0.01%	
acetone	812.8	9.28±0.02%	6.06±0.10%	10.11±0.12%	Pungent
2-Octanone	1304.7	3.42±0.03%	2.97±0.12%	0.78±0.04%	Fat, Fragrant, Mold
3-Octanone	979.1	0.72±0.02%	0.72±0.06%	0.28±0.02%	Butter, Herb, Mold
pent-1-en-3-ol	1156.3	1.39±0.06%	1.40±0.06%	1.05±0.07%	Fruit fragrance, vegetable fragrance
1-Butanol	1116	0.36±0.02%	0.37±0.02%	0.35±0.01%	Fruit
1-Propanol	1023.9	0.99±0.02%	0.69±0.02%	0.41±0.02%	Alcohol, Candy, Pungent
1-Hexanol	873.6	0.23±0.16%	0.63±0.12%	0.33±0.09%	Banana, Flower, Grass, Herb
Ethanol	948.2	7.29±0.01%	6.92±0.03%	4.98±0.02%	Alcohol, sweet
1-pentanol	1242.4	0.35±0.02%	0.29±0.02%	0.89±0.04%	Balsamic, Fruit, Green, Pungent, Yeast
2-Butanol	1008.8	0.64±0.01%	0.10±0.01%	0.15±0.01%	Wine
3-methyl-3-butenol	1245	0.72±0.04%	0.78±0.02%	0.62±0.04%	
2-Butoxyethanol	906.2	0.95±0.12%	0.92±0.04%	0.45±0.03%	
3-heptanol	1304.1	0.40±0.02%	0.33±0.02%	0.15±0.00%	Herb
			Esters		
2-Phenyl ethyl acetate	1240.9	1.27±0.06%	1.17±0.05%	4.90±0.43%	Fruit
Ethyl benzoate	1155.4	1.80±0.03%	1.29±0.05%	3.64±0.00%	Bitterness
butyl-2-methylbutanoate	1041.3	0.40±0.09%	0.70±0.01%	0.83±0.10%	Fruit fragrance
Ethyl Acetate	871.5	0.38±0.05%	0.43±0.01%	0.31±0.02%	Aromatic, Brandy, Grape
methyl hexanoate	932.6	0.47±0.09%	0.82±0.08%	0.84±0.22%	Pineapple
Ethyl pentanoate	902	3.03±1.85%	6.61±0.87%	3.92±0.80%	Apple
n-Pentyl acetate	913.3	0.14±0.06%	0.39±0.06%	0.33±0.10%	Pineapple, Apple
Isopentyl formate	796.6	0.09±0.00%	0.13±0.01%	0.07±0.00%	Apple
iso-Propyl acetate	857.2	0.32±0.02%	0.36±0.02%	0.12±0.00%	Banana
methyl acetate	801.4	0.48±0.01%	0.79±0.02%	0.22±0.02%	Mint, Cool
Ethyl lactate	794.1	0.29±0.01%	0.34±0.00%	0.09±0.00%	Cheese, Floral, Fruit, Pungent, Rubber
ethyl 2-hydroxypropanoate	807.8	0.07±0.00%	0.21±0.00%	0.03±0.00%	Rum, fruit, and creamy aroma
ethyl trans-2-hexenoate	1045	6.42±1.80%	12.46±1.02%	18.81±3.49%	Fruit
			Acids		
Acetic acid	1432	9.01±0.55%	7.97±0.03%	6.71±0.03%	Acid, Fruit, Pungent, Sour, Vinegar
cis-3-Hexenyl lactate	1234.7	1.74±0.19%	2.43±0.03%	4.60±0.35%	Green
			Ethers		
Dibutyl sulfide	1069	2.61±0.02%	3.68±0.01%	0.45±0.02%	Green
dimethyl sulfide	770.2	2.11±0.03%	3.78±0.04%	0.90±0.01%	Seafood flavor
			Enolefins		
Undecane	1079.1	0.48±0.01%	0.47±0.01%	0.25±0.02%	
2,2,4,6,6-Pentamethylheptane	977.7	0.65±0.01%	0.37±0.03%	1.41±0.05%	

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2,2,4,6,6-Pentamethylheptane-M	994	7.68±0.06%	5.04±0.25%	4.21±0.02%	
beta-Pinene	977	1.02±0.01%	1.15±0.00%	0.88±0.02%	Pine, Polish, Wood
o-Xylene	888.3	0.52±0.16%	0.21±0.03%	0.75±0.12%	Fragrance
acrylonitrile	1001.6	2.21±0.01%	0.90±0.01%	1.87±0.01%	Peach kernel flavor
2-acetyl-1-pyrroline	926.8	12.94±0.31%	9.01±0.35%	6.81±0.33%	Savory
Styrene	866	0.08±0.01%	0.09±0.01%	0.14±0.03%	Special aroma
			Heterocyclic		
tetrahydrofuran	887.2	0.61±0.12%	0.50±0.04%	1.06±0.21%	Ethyl ether flavor
Methylpyrazine	1244.7	3.37±0.03%	2.93±0.05%	8.02±0.00%	Nuts
2-pentylfuran	1214.9	2.04±0.07%	2.09±0.09%	0.47±0.04%	Butter, Floral, Fruit, Green Bean
Pyridine	1174.7	1.21±0.07%	0.77±0.01%	0.47±0.00%	Odor
2-acetyl-3-methylpyrazine	1074.1	0.30±0.01%	0.18±0.00%	0.11±0.00%	Wood fragrance
2,5-Dimethylthiophene	1168.6	0.61±0.02%	0.50±0.01%	0.43±0.00%	

To enhance characterisation of varying volatile compounds, we calculated the relative difference among the flavour compounds in the three samples based on signal intensity on the fingerprint, demonstrated in Figure 5. The similarity of the acquired VOC fingerprints was assessed using statistical approaches like the heat map and clustering method, as detailed in Figure 5a.The volatile organic compounds (VOCs) present in three samples of walnut purple rice yoghurt were analysed and vertically clustered. Figure 4 displays the obtained results. Based on Figure 5b, distinct variations in the contents of H1, H2 and C1 can be observed among the three samples. From the figure, it is apparent that the primary components of walnut purple rice yogurt are H1 (with 25.57% olefins, 15.15% esters, 14.12% ketones, and 13.31% alcohols), followed by H2 (with 25.69% esters, 17.26% olefins, 12.43% alcohols, and 10.71% ketones), and C1 (with 34% esters). 11% olefins, 16.32% alcohols, and 11.31% acids were detected in Walnut Purple Rice Yoghurt H1. The content of 2-acetyl-1-pyrroline was the highest in this product, and the threshold for this compound was lower, which gives it a savoury taste and contributes to the overall flavour [40]. Compared to H2, the proportion of olefins in walnut purple rice yoghurt H1 significantly increased, with a concurrent increase in ketone content. The ketones mainly resulted from microbial metabolism during fermentation [8],It is produced mainly by the oxidation of esters and unsaturated fatty acids [26],Many of these ingredients have a pleasant aroma, possibly accounting for the reduction in relative ester levels in H1 walnut purple rice yoghurt: the majority of ketones had a high threshold and did not significantly affect the flavour profile [25]. Fermentation can alter the composition and proportions of volatile flavour compounds, leading to a more intense flavour profile. A combination of flavour compounds give rise to the characteristic flavour of Walnut Purple Rice Yoghurt H1. In comparison to regular temperature yoghurt C1, esters exhibit the greatest disparity in their relative content between the two. The relative content of esters in plain normal temperature yoghurt C1 is twice as high as in H1 yoghurt. Esters are key components of food flavour.As well as giving foods a special 'floral and fruity flavour', it can also mask the 'unpleasant, irritating taste' caused by free fatty acids [15],In contrast, the proportion of aldehydes and ketones present in Walnut Purple Rice Yoghurt H1 exceeds that of regular yoghurt C1. In contrast, the proportion of aldehydes and ketones present in Walnut Purple Rice Yoghurt H1 exceeds that of regular yoghurt C1. Aldehydes and ketones constitute the primary volatile compounds of Walnut Purple Rice Yoghurt H1, which impart a distinctive scent in plants. Aldehydes primarily arise from the oxidation of lipids [23],The threshold is generally low, with a fragrant and fruity taste, which also gives H1 a different flavour from ordinary C1 yoghurt at room temperature.

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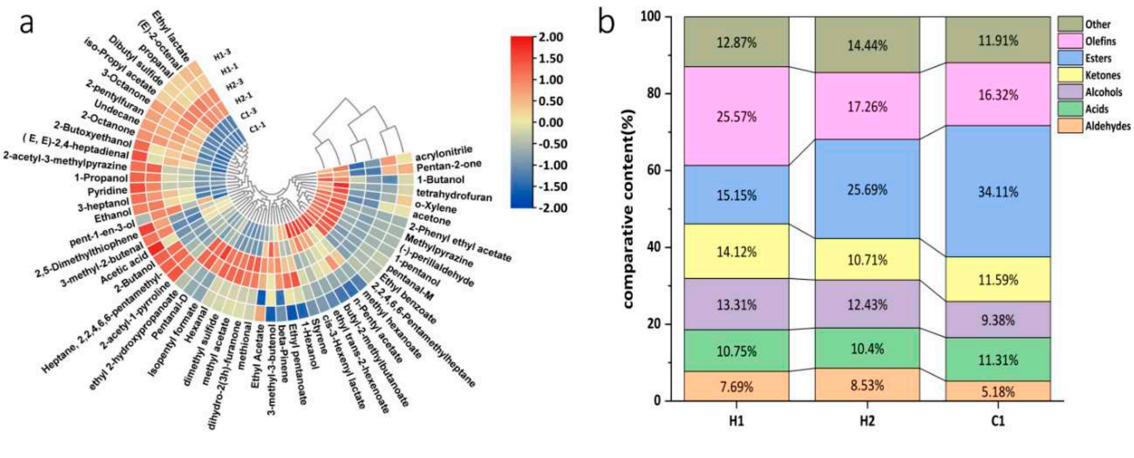

Figure 5. Cluster heat map (a) and difference map (b) of relative content of volatile flavor substances in samples.

3.4.3. Principal component analysis of volatile flavor compounds

Principal Component Analysis (PCA) is a multivariate statistical approach that diminishes the dimensions of a dataset composed of numerous related variables, at the same time preserving as much variation within the data as possible. This method has garnered broad usage in investigating variations in food aroma compounds [27]. Principal component analysis was conducted on the peak intensities of the volatile compounds in the three specimens, and Figure 4 displays the findings. The first principal component PC1 made a contribution rate of 59.5%, while the second principal component PC2 contributed 30.3%. The combined contribution rate of the first and second principal components was 89.8%, containing the majority of the information on the three samples and presenting the primary features of the volatile aroma. The principal component analysis chart illustrates that the three sample groups were significantly separated, denoting GC-IMS technology utilization in the identification of the volatile compounds in the samples. When combined with PCA, the samples are better distinguished. Simultaneously, the findings reveal significant contrasts between the three samples.

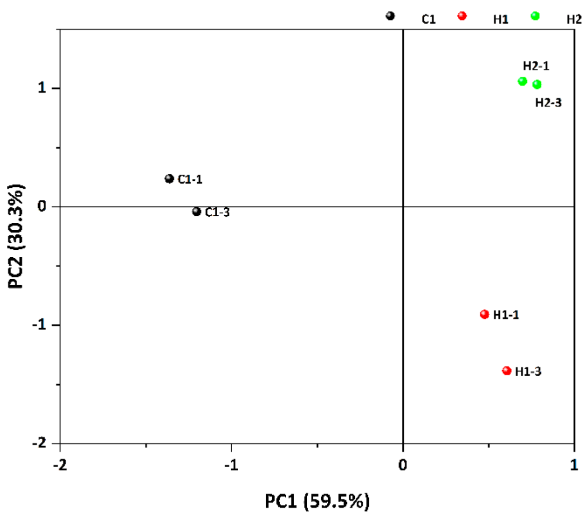

Figure 6. PCA analysis diagram of volatile substances in three samples.

3.5. Organoleptic investigation

The sensory evaluations of three samples at varying levels of ripeness were analysed using an OPLS-DA score plot. The results can be seen in Figure 7. The associated model was generated by

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randomly shuffling the order of categorical variables, and yielded values of R2X = 0.699 and R2Y = 0.179. These R2 and Q2 values indicate a high level of general interpretation for the model when they fall within the range of 0.5 to 1 [34],Q2 = 0.965 suggests excellent predictive ability of the model. Clear stability and reliable predictability indicate that the model meets all the necessary requirements to differentiate sensory differences of the three samples. To check for overfitting, we disrupt the categories of some samples, and perform 200 instances of replacement fitting. Figure 5 demonstrates that R2 and Q2 intersect with the longitudinal axis at (0, -0.0068) and (0, -273) respectively. Additionally, the slopes of the two regression lines for R2 and Q2 are relatively high. The values for R2 and Q2 produced by the random arrangement on the left exhibit lower values compared to those on the right. Additionally, the fact that the Q2 regression line has a negative intercept suggests that the OPLS-DA model does not exhibit overfitting and demonstrates reliable predictive capabilities. This renders it suitable for discriminant analysis across diverse samples. Table 4 reveals that fermentation has significantly shifted the taste and flavour of walnut purple rice yoghurt. The bitterness, sweetness, and astringency of H1 were significantly decreased ($p < 0.05$). In line with expectations, the fermented and sour tastes received the highest scores. Fermentation enhances food acidification, augmenting the sensory, nutritional, textural, and microbial safety of food. Furthermore, it negates the use of chemical preservatives and maintains the original natural state of the food. In our study, while fermentation significantly decreased the off-flavours of nuts and grains, it did not lower the unpleasant levels, which could be attributed to the new compounds generated by microbial fermentation [33]. Fermentation significantly enhanced the flavour, as well as the dispersibility and adhesion of the yoghurt, while reducing its graininess. The improved taste may be attributed to the fermentation process, which results in a smoother taste compared to the walnut purple rice milk. The overall acceptance of the walnut purple rice yoghurt is comparable to that of regular room temperature yoghurt. Although the flavour of walnut and purple rice yoghurt is slightly less superior to that of traditional yoghurt, its unique nutty and cereal notes add distinctive qualities. Moreover, team members rate it similarly to typical yoghurt in terms of acceptance and degree.

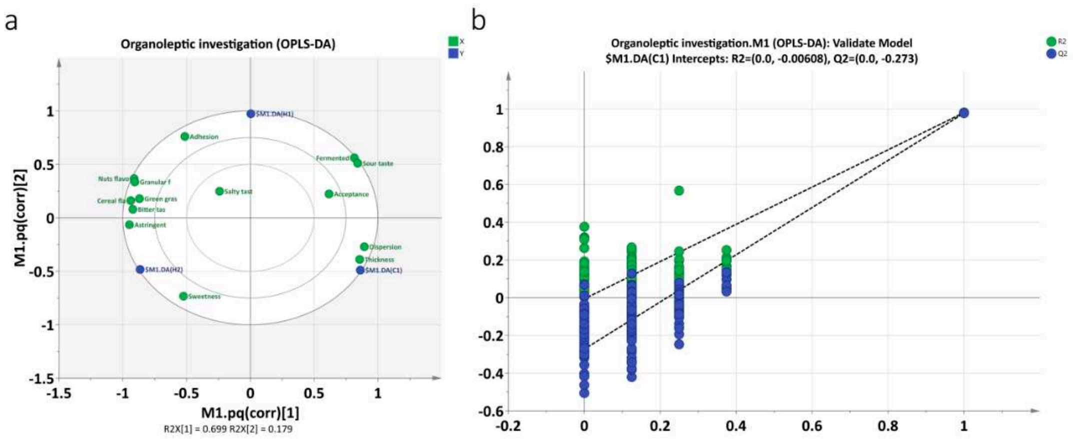

Figure 7. OPLS-DA and its fitting curve of three samples.

Table 4. Scores of 14 sensory attributes of three samples.

		C1	H1	H2
Flavor	Green grass flavor	1.25±0.62[c]	3.42±0.67[b]	4.50±1.00[a]
	Nuts flavor	0.00±0.00[c]	4.17±0.72[b]	5.00±0.74[a]
	Grainy flavor	0.00±0.00[c]	2.17±0.58[b]	3.33±0.65[a]
	Fermented flavor	6.50±0.80[b]	7.17±0.58[a]	0.00±0.00[c]
Taste	Sweetness	5.83±0.58[b]	4.83±0.72[c]	7.25±0.45[a]
	Sour taste	5.83±0.94[a]	5.92±0.67[a]	0.00±0.00[c]
	Bitter taste	0.33±0.49[c]	1.92±0.51[b]	3.17±0.58[a]

	Salty taste	0.42±0.51[b]	0.83±0.39[a]	0.67±0.49[ab]
	Astringent taste	0.25±0.45[c]	1.75±0.45[b]	3.75±0.62[a]
Textural attributes	Dispersion	7.25±0.45[a]	4.50±0.52[b]	3.67±0.78[c]
	Thickness	6.92±0.51[a]	3.67±0.49[b]	4.08±0.67[b]
	Adhesion	4.08±0.51[c]	6.50±0.67[a]	5.50±0.52[b]
	Granular feeling	0.00±0.00[c]	4.00±0.60[b]	4.83±0.72[a]
Overall	Acceptance	7.17±0.58[a]	6.92±0.51[a]	6.08±0.67[b]

[a-c] Means within a row with different superscripts are different at $P < 0.05$.

4. Conclusions

Plant-based yoghurt and conventional yoghurt are created using contrasting raw components. Conventional yoghurt is produced through fermentation of bacterial strains in animal-based raw materials like milk or goat's milk. In contrast, plant-based yoghurt is produced using plant-based materials including soybeans, coconuts and nuts. There is a growing demand for plant-based yoghurt as more consumers prefer it. While there have been numerous studies on the features and advancements of plant-derived yoghurt, research into the comparisons between plant-derived yoghurt and traditional yoghurt has been limited. This study aimed to compare the differences between walnut purple rice yoghurt, before and after fermentation, and conventional dairy-based yoghurt. The results revealed that walnut purple rice yoghurt exhibited high pseudoplasticity and shear-thinning non-Newtonian fluid properties. Similar to regular dairy yoghurt, walnut purple rice yoghurt exhibits a semi-solid gel-like structure with good viscosity and strong recovery properties. Notably, there are significant differences in flavour and taste between walnut purple rice yoghurt before and after fermentation and traditional dairy yoghurt. However, the use of electronic nose, electronic tongue, GC-IMS and sensory analysis reveals that walnut purple rice yogurt is better received. This provides a theoretical basis for the growth of plant-based yogurt and the industrialisation and in-depth processing of walnuts.

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Institutional Review Board Statement: The study was reviewed and approved by the Yunnan Agricultural University IRB.

Informed Consent Statement: Informed consent was obtained from each subject prior to their participation in the study.

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

Conflicts of Interest: The authors declare no competing financial interest.

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