# Testing consumer perception for a new Australian wine product for the Australasian market: sensory properties, chemical composition and consumer acceptance of Shiraz wine containing Ganoderma lucidum.

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# THESIS SUMMARY

*Ganoderma lucidum* (*GL*) is a wood-degrading basidiomycete. It is well-known as a health-promoting mushroom in "herbal" oriental medicine, and has been used for thousands of years owing to the belief that it prevents and ameliorates a range of human diseases. Recently, there has been an observed increase in usage of *GL* due to the development of multiple, off-the-shelf *GL*-based products such as tea, and dietary supplements (powders, extracts, oils, and so on). This provides the inspiration to develop additional products containing *GL* such as those involving wine, which could be targeted at Asian markets. China is now Australia's largest wine export market, and along with emerging wine markets in other Asian countries, this offers Australia a unique opportunity. Taking advantage of both its proximity to these markets and its clean and safe food product image, Australia has the prospect to capitalise on the concept of a grape wine containing *GL* extracts. However, there is an absence of research related to developing novel *GL* wine products and targeting Australian wine exports to new markets such as China, or Australasia in general. Development of such products requires understanding of the acceptance of these novel wines in the prospective markets, the underlying sensory and chemical properties, and consumer wine taste preferences. This thesis consists of a number of original studies aimed at investigating 1) Australian, Chinese and Vietnamese consumer perceptions and attitudes towards products including Australian Shiraz wines enriched with *GL*, 2) generating prototype Shiraz red wines either fermented from grape juice in the presence of *GL* extracts or produced with extracts added to the wine post-fermentation and 3) undertake compositional measures, sensory analyses and a consumer preference study. The research findings have been presented as a series of manuscripts after the first chapter, an initial review of the *GL* literature.

As a first step, to understand consumers' perceptions and attitudes towards a novel Australian Shiraz wine with *GL,* an online survey was undertaken to investigate Australian,

Chinese and Vietnamese consumers' knowledge of *GL*, products containing *GL,* and acceptance of a novel *GL* wine. Importantly, the results presented in Chapter 2 augers well for a new Australian *GL* containing grape wine, as they revealed that all consumer groups accepted the notion that *GL* wine products would be worth tasting and they would try them at social events, with Vietnamese consumers being especially interested. Wine neophobia was measured using the wine neophobia scale to examine if this was an underlying factor in consumer acceptance and willingness to try these wines across the three nationalities. Three segments containing wine neophiles (n = 110), neutrals (n = 190) and wine neophobes (n = 112) were identified and the study showed that compared to the Vietnamese group, Australian and Chinese participants were significantly more wine neophilic. Moreover, neophiles were more prepared to taste and purchase *GL* wine products compared to neophobes in all countries.

The promise of potential demand for these *GL* wine products in specific markets (Asian countries) where most consumers are more likely familiar and have a strong belief in the potential health benefits of *GL,* led to a study aimed at developing novel Australian Shiraz red wine products enriched with *GL* extracts as detailed in Chapter 3. Shiraz is Australia's most planted wine grape variety but is also an important red grape variety globally. Furthermore, red wine is a popular wine of choice in China as red is associated with good luck. If successful, a novel Shiraz *GL* red wine product could boost Australian wine exports. The study undertook several preliminary experiments of small-, and medium-scale fermentations (100 mL and 5 L, respectively) to determine the *GL* levels suitable for larger-scale fermentation (28 L, used for chemical and sensory analysis) where *GL* was added prior to or after primary fermentation. Due to *GL*'s known anti-microbial properties, the presence of these extracts impacting on yeast fermentation kinetics was examined. As the presence of *GL* extract in the ferment could potentially act as a precursor source for yeast derived volatile aroma compounds, the basic and volatile chemical composition and sensory properties of the resulting wines were profiled. In the small-scale ferments, red grape juice or chemically defined grape juice media fermentation

kinetics were not significantly different between *GL* treatments (0, 4.5, 9, 18 and 36 g/L) or non-*GL* controls. Similarly, wines made from larger-scale fermentation with *GL* added 0, 1, 2 and 4 g/L were considered residual sugar and malic acid dry, indicating that the treatments successfully underwent primary and malo-lactic fermentations. As a basic tenet, consumer science has not used consumers to provide objective, sensory measures in food and beverages. Recent developments have shown that consumers are able to complete these tasks if provided with an appropriate method. Furthermore, analytical tasks and hedonic ratings can be combined without biasing liking results. As such, a Rate-All-That-Apply (RATA) (n = 65) consumer sensory panel was used to investigate the influence of *GL* extract additions on wine sensory characteristics and liking. Thirty-nine out of 54 sensory attributes assessed were significantly different among the wines but no sensory differences were observed between pre- or post-addition wines. In general, wines at the highest level of added extract (4 g/L) were more complex, savoury, woody, toasty, with tobacco, mushroom, pepper and earthy aromas, pepper, green and mushroom flavours, higher astringency and roughness and bitter taste. Wines made with 2 g/L *GL* were more herbaceous, with green capsicum, peppery, spicy and jammy notes. While wines with the lowest level of *GL* (1 g/L) were described as having more red fruit, floral and confectionery aromas and flavours, smooth mouthfeel and sweet taste. Increasing levels of *GL* extract resulted in significant differences between samples with only ethanol, colour parameters and residual sugar differences likely to have a noticeable impact. The volatile compounds 2-phenylethanol, ethyl acetate, limonene, 1-octanol and hexanoic, octanoic, decanoic and 3-methylbutanoic acids, were found to differ significantly between the treatments. Unlike Principal Component Analysis (PCA), a clear separation was evident in the Partial Least Squares regression (PLS) plot between *GL* wine groups, including pre- and post-fermentation wines. Specific volatiles were also found to be correlated with relevant sensory attributes in wines, e.g. mushroom aromas and 1-octanol; red fruity aromas and octanoic and decanoic acids. These study findings indicated that the use of *GL* in the winemaking process could generate

wines differing in sensory profiles, which could appeal to the palates of consumers in Asian countries.

It is widely accepted that consumer wine choice, liking and re-purchase depends largely upon wine taste, aroma and flavour. To examine the drivers of wine consumer liking of novel Australian Shiraz *GL* wine products, a sub-set (n = 6) of the study wines was chosen by interpreting the liking responses of the RATA panel mentioned in Chapter 3. Wine consumers (n = 124) at a central location (food market in Adelaide, South Australia) were asked to complete a questionnaire followed by participating in a blind tasting of the six wines, for collection of their hedonic responses. This data was merged with the wine chemical composition and sensory attribute data for analysis and results presented in Chapter 4. Three hedonic clusters were identified; cluster 1 (C1, n = 41), cluster 2 (C2, n = 37) and cluster 3 (C3, n = 46). PCA was performed on the sensory data with the hedonic clusters as supplementary data, revealing a clear separation between hedonic clusters. C1 preferred red appearance, pepper, red fruit, cooked vegetable, earthy, mushroom, leather and woody aromas, and pepper, green capsicum, spice, cooked vegetable, mushroom and floral flavours, and more astringent mouthfeel in wines without and with 2 g/L *GL* added pre-fermentation (PRE 2) and. In contrast, C2 liked brown appearance and tobacco aromas in wines made with 1 g/L (PRE 1 and POST 1) and 4 g/L *GL* added post-fermentation (POST 4). C3 liked wines made with 1 g/L *GL* and control wines that had floral and confectionery aromas and red-fruit notes with a smooth mouthfeel. The sensory attributes and volatile flavours that significantly differentiated the wines were subjected to PLS regression, which indicated the important positive and negative drivers of liking amongst the hedonic clusters.

The results of the overall study have provided significant new knowledge regarding the perceptions about *GL* wine products of consumers in Australia's domestic, key export and emerging wine markets. Results also indicated that the development of a unique Australian wine product concept containing *GL* could open a new niche wine product category with diverse

flavour profiles to satisfy the tastes of a number of consumer segments. Understanding the flavours and chemical profiles of these wines, and those that drive consumer preferences, could guide wine producers in the creation of novel wine products that are not only palatable but meet the expectations of the target wine markets.

# DECLARATION

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in my name, in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. In addition, I certify that no part of this work will, in the future, be used in a submission in my name, for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint-award of this degree.

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Anh Ngoc Hoang Nguyen

**1st October 2019**

# PUBLICATIONS

This thesis consisted of two manuscripts published in Food Research International (FRI, a journal of Canadian Food Science and Technology for the special issue titled *Global Perspectives on Food and Consumer Science: A cross-cultural approach*) and Foods (for the special issue titled *Chemical and Sensory Analysis of Alcoholic Beverages*). The FRI journal and had an impact factor of 3.6 in 2018 according to InCites Journal Citation Reports. One additional manuscript was submitted to a scientific journal: Foods (for the special issue titled *Novel foods and Nutritional Function*).

The publications included in the thesis were:

Chapter 2 Nguyen, A. N. H.; Johnson, T. E.; Jeffery, D. W.; Danner, L.; Bastian, S. E. P., A cross-cultural examination of Australian, Chinese and Vietnamese consumers' attitudes towards a new Australian wine product containing *Ganoderma lucidum* extract. *Food Research International* **2018,** *115*, 393-399. *doi.org/10.1016/j.foodres.2018.10.086*

Chapter 3 Nguyen, A. N. H.; Capone, D. L.; Johnson, T. E.; Jeffery, D. W.; Danner, L.; Bastian, S. E. P., Volatile composition and sensory profiles of a Shiraz wine product made with pre- and post-fermentation additions of *Ganoderma lucidum* extract. *Foods*, **2019***, 8 (11), 538-554. doi: 10.3390/foods8110538.*

The following chapter formatted as a manuscript and submitted to a scientific journal is:

Chapter 4 Nguyen, A. N. H.; Johnson, T. E.; Jeffery, D. W.; Capone, D. L.; Danner, L.; Bastian, S. E. P., Sensory and chemical drivers of wine consumers' preference for a new Shiraz wine product containing *Ganoderma lucidum* extract as a novel ingredient. *Foods*, **2019**, Submitted.

# CONFERENCES

**1. School of Agriculture, Food & Wine, Waite Campus, The University of Adelaide–Postgraduate Symposium, 27–28 September 2016, Adelaide, South Australia.** Presented a talk titled "Testing consumer perception for a new Australian wine product for the Chinese and Vietnamese markets: Biological activity, sensory properties and consumer acceptance of *Ganoderma* wine product".

**2. Eurosense–A Sense of Taste, the Eight European Conference on Sensory and Consumer Research, 2–5 September 2018, Verona, Italy.** Presented a poster titled "Sensory profile and consumers' perception and consumption behaviour of a novel Australian Shiraz wine product with *Ganoderma lucidum* extract".

**3. TMedPM–First International Conference on Traditional Medicine, Phytochemistry and Medicinal Plants, 15–17 October 2018, Narita, Japan.** Presented a talk titled "Utilising grape marc for the cultivation of *Ganoderma* strains of medicinal mushrooms in Australia".

**4. Crush–The Grape and Wine Science Symposium, 25–26 September 2018, Adelaide, South Australia.** Presented a poster titled "Understanding wine consumer's acceptance and preference for a new Shiraz wine".

**5. AWITC–The 17$^{th}$ Australian Wine Industry Technical Conference & Trade Exhibition, 21–24 July 2019, Adelaide, South Australia.** Presented a poster titled "A new Australian Shiraz wine product containing *Ganoderma lucidum* extract: chemical composition, sensory profiles and wine consumer acceptance".

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# ABBREVIATIONS

| | |
|---|---|
| ANOVA | Analysis of Variance |
| C1, C2 and C3 | Cluster 1, Cluster 2 and Cluster 3 |
| CATA | Check-all-that-apply |
| CDGJM | Chemically Defined Grape Juice Media |
| CIELAB | International Commission on Illumination |
| DAP | Diammonium phosphate |
| FNS | Food Neophobia Scale |
| g/L | gram per litre |
| GC-MS | Gas chromatography-mass spectrometry |
| *GL* | *Ganoderma lucidum* |
| hL | hectolitre (100 L) |
| HS-SPME | Headspace-solid-phase micro-extraction |
| LSD | Least significant difference |
| LTM | Low thermal mass |
| *m/z* | Mass-to-charge ratio |
| min | Minute |
| MLF | Malolactic fermentation |
| NPD | New product development |
| PC1 | Principal Component 1 |
| PC2 | Principal Component 2 |

| | |
|---|---|
| PCA | Principal Component Analysis |
| PLS | Partial Least Squares |
| PMS | Potassium metabisulphite |
| POST | Post-fermentation |
| PRE | Pre-fermentation |
| RATA | Rate-all-that-apply |
| RGJ | Red grape juice |
| RI | Retention index |
| RS | Residual sugar |
| *S. cerevisiae* | *Saccharomyces cerevisiae* |
| TA | Titratable acid |
| VA | Volatile acidity |
| WNS | Wine Neophobia Scale |
| YAN | Yeast assimilable nitrogen |
| YEPD | Yeast extract, Peptone and Dextrose Media |
| LSC | Liquid-state cultivation |
| SSC | Solid-state cultivation |
| LSF | Liquid-state fermentation |
| SSF | Solid-state fermentation |

# Chapter 1

# Literature review

This literature review was prepared within the first year of candidature and comprised the literature up to October 2016. The directions of the project changed due to early difficulties obtaining sufficient *Ganoderma lucidum (GL)* material from culturing mycelia and the cultivation of fruiting bodies. Further research on examining the bioactivities of *GL* extract on gastrointestinal cells and cultivation of *GL* on different substrates including wine grape marc, was not pursued nor presented in this thesis. As a consequence, the research still examined *GL* wine production but more emphasis was placed on consumer studies, which is reflected in the aims and objectives of the project which, since 2016, have been updated. Any relevant additional literature, particularly around consumer preferences, behaviour and new product development, has been included in the introduction sections of Chapters 2 to 4.

## 1. Introduction

*Ganoderma lucidum* (*GL*) is a wood-degrading basidiomycete, that has been well known as a natural "herbal" or oriental medicine. Called Lingzhi in China, Reishi in Japan, Yeongji in Korea, and Linh chi in Viet Nam, *GL* is conventionally recognised as a powerful medicinal fungi when it is consumed as a special tea (Sanodiya, Thakur, Baghel, Prasad, & Bisen, 2009) or mixed with traditional brandy (Pecic et al., 2012) to achieve greater vitality and longer life span (Bishop et al., 2015; Boh et al., 2007; Fraga et al., 2014; Leskosek-Cukalovic et al., 2010; Paterson, 2006; Sanodiya et al., 2009; Shiao, 2003; M. Yang et al., 2007). *GL* has been used for thousands of years (Chien, Ho, Chiang, & Hwang, 2011) and commonly consumed in East Asian countries (Lindequist, Julich, & Witt, 2015), Southeast Asia, and China (C. Li et al., 2015). There are many researchers worldwide studying the therapeutic effects of *GL* in the treatment of different health problems and diseases (Jong & Birmingham, 1992), including chronic hepatopathy and hypertension (Boh et al., 2007), gastric ulcers (Sanodiya et al., 2009), diabetes (Ma, Hsieh, & Chen, 2015; Seto et al., 2009), HIV (Boh et al., 2007; Gorases & Goraseb, 2013; Sanodiya et al., 2009), and cancer (Bone, 2007; Wachtel-Galor, Yuen, Buswell, & Benzie, 2011; Zaidman, Yassin, Mahajna, & Wasser, 2005; Lin Zhang, Reddy, & Koyyalamudi, 2014), to the extent that *GL* is widely considered as a popular remedy to promote health (Jong & Birmingham, 1992; Shiao, 2003).

According to Wachtel-Galor et al. (2011), a large number of products containing *GL* are offered in different formulations; such as powders, dietary supplements, foods and drinks produced from different parts of the mushroom, including *GL* mycelial medium, spores and fruiting body (Leskosek-Cukalovic et al., 2010; Wachtel-Galor et al., 2011). These are then marketed as "health food" or "health drinks" or *Ganoderma*-based products. Although *GL* has

been used in the most simple way as tea for a very long time[1], the presence of *GL* in Asian markets recently opened new trends of enriched products, e.g., coffee (Ganocafé), chocolate (Ganochocolate), toothpaste (Ganofresh), soap (Ganosoap), or functional foods (Gano-daily vegetable capsules). Hence, the potential health benefits offered by *GL* have led to an increase in the variety of *Ganoderma*-based products sold in Asian markets under different names: dietary supplements, functional foods, mycopharmaceuticals, and designer foods including probiotics and prebiotics (Wasser, 2014).

Although there have been an increasing number of companies (Ganoexcels, ganoorganics) trading their *GL*-based products around the world, the evaluation of behaviour and cultural attitudes that determine why Asian consumers would purchase such functional foods and beverages is lacking. This could be extended to other products enriched with *GL* such as wine, and ultimately lead to the development of new wine products. Currently, *GL* extract is not a legal additive for beverages but that does not prevent it from being explored. For example, in Korea *GL* extracts were applied during the process of baking bread in order to enhance the perceived health products' texture (Chung, Lee, & Kwon, 2004). *GL* was also used to enhance the quality and functionality of traditional Korean Yakju wine by using it in the fermentation (J. H. Kim, Lee, Lee, Choi, & Lee, 2004). In addition, Pilsner beer in Serbia with *GL* aseptically added, created a beer with a better perceived body and health effects (Leskosek-Cukalovic et al., 2010). However, there is no *GL* enhanced wine produced in Australia. There are several reasons behind wanting to create *GL* enhanced wines in Australia. Firstly, it could stimulate success in Asian wine markets in terms of new wine product development strategies and offerings. Secondly, customers will have several potential benefits from consuming wines with *GL* that may more closely align with their needs and

[1] The conventional way is for Asian consumers to use *GL* fruiting bodies ground into fine powder and soaked in hot water.

expectations. Developing such a wine product could provide a competitive edge and create new wine market opportunities.

## 2. Literature review

### *2.1 GL morphology and taxonomy*

#### 2.1.1 Morphological characteristics

*Ganoderma* species are found all over the world (in tropical and subtropical areas (Sanodiya et al., 2009)) and display diverse characteristics, such as differently coloured basidiocarps (fruiting body) (Figure 1) which can range through red, black, brown, white, yellow, or purple (Jong & Birmingham, 1992). The red variety is the most common of the varieties commercially cultivated in North America, China, Taiwan, Japan and Korea (Sanodiya et al., 2009). The shape of *GL* fruiting bodies which are circular, semi-circular, fan-shaped or kidney-shaped (Figure 1 shows kidney-shaped forms of *GL* fruiting bodies), vary based not only on the species, but also on the host, nutrients and environments. The stipe is lateral and rarely eccentric. An important part of the *Ganoderma* species for completing its life cycle is spore, which is white at first and becomes light brown during the later stage of the cycle (Chang & Miles, 2004).

**Figure 1.** Two fruiting bodies of *GL* showing its common kidney shape (Chang & Miles, 2004)

According to Sanodiya et al. (2009), the morphology of *GL*'s fruiting bodies changes at different growth stages when observing *GL* during artificial cultivation (Figure 2). In the initial stage of primordia formation to further growth of the cap, young *GL* fruiting bodies have a red, bright yellow and white surface (Figure 2J and 2K). Later, in the mature stage, the white and yellow shades disappear and there is only the presence of a varnished, and reddish to reddish brown surface, resulting in quite beautiful and distinctive *GL* fruiting bodies (Figure 2L).

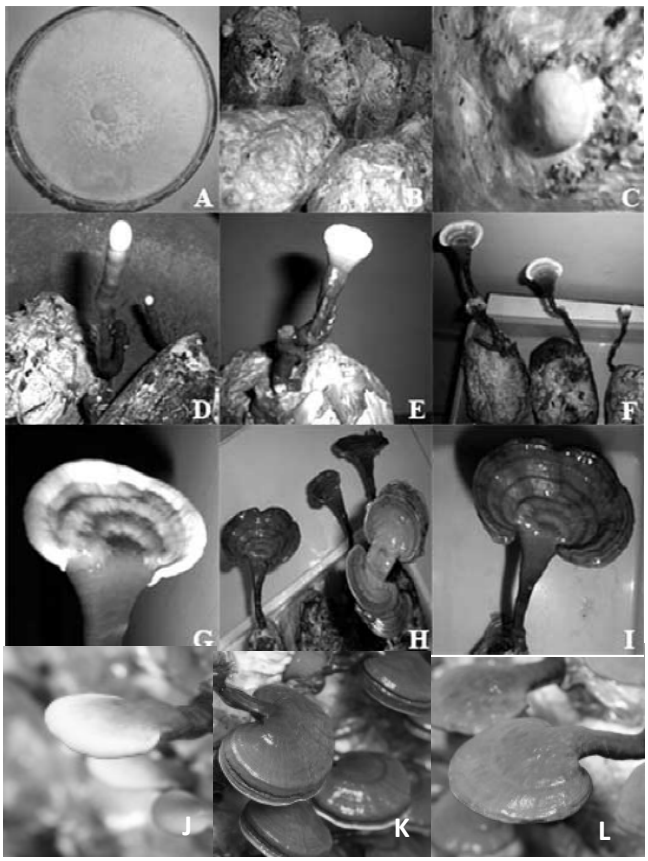


**Figure 2.** Different growth of *GL* during artificial cultivation (Sanodiya, Thakur, Baghel, Prasad, & Bisen, 2009)
*A: culture plate, B: Mycelial colonization on solid substrate, C: Primordia formation, D: Elongation of Primordia, E and J: Cap formation, F: Flattening and growth of cap, G: thickening of cap, H and K: Maturation of fruiting body, I and L: Mature fruiting body.*

In the summer and autumn, the fruiting bodies of *GL* release countless spores into the air. These spores are blown to new locations and when they encounter favourable conditions (temperature, humidity, light, growth substrates and so on), spores will germinate to create hypha. Then, the life cycle of *GL* (Figure 3) subsequently starts when multiple hyphae fuse

together, resulting in a network of threads called mycelium. The next stage is the formation of a fruiting body. The mature fruiting body creates spores on the pileus (Figure 1) and the life cycle of *GL* repeats.

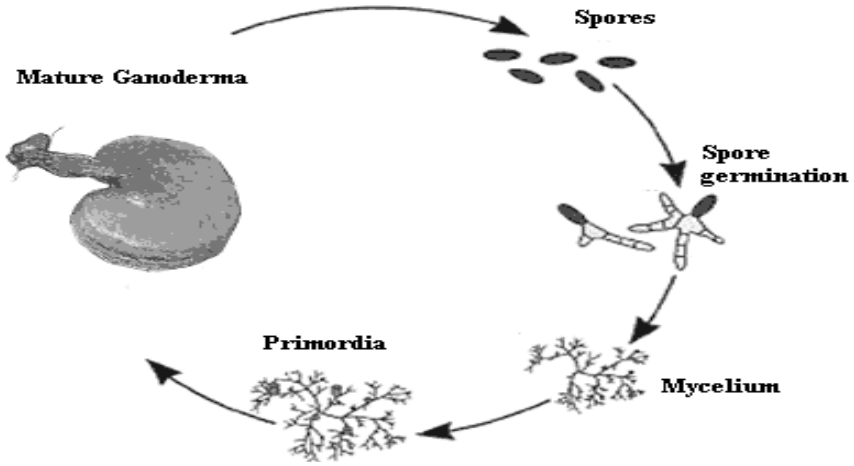


**Figure 3.** *GL* life cycle (mushroompalace.com)

2.1.2 Taxonomy

During the 1880s the identification of the genus *Ganoderma* was mainly based on host specificity, geographical distribution, and macro-morphological features of the body. The last identification consists of two, including context colours that vary from white to deep brown, and the shape of the margin of pileus (Figure 4). These two features are characteristics that have historically been useful for classification (Flood et al., 2000). The genus *Ganoderma* was originally established by Finnish mycologist Petter Adolf Karsten in 1881 and later, the name "*Ganoderma lucidum* (Curt.: Fr.) P. Karst" has been popularly cited in research papers from that time until the present day (Roberts, 2004). Also, according to Karsten, this genus was divided into two distinct groups: laccate pilei (*GL* complex) and non-laccate pilei (*Ganoderma applanatum* complex). The former features annual fruiting bodies having a yellow to reddish laccate (waxy or lacquer-like) cuticle and an upper layer that is smooth or often concentrically zoned and grooved. The latter is described with the fruiting bodies being perennial with a brown to black cuticle with the upper layer of the fruiting body composed of

a hard surface crust that is usually cracked, furrowed, and ridged but not varnished (Figure 2L) (Chang & Miles, 2004).

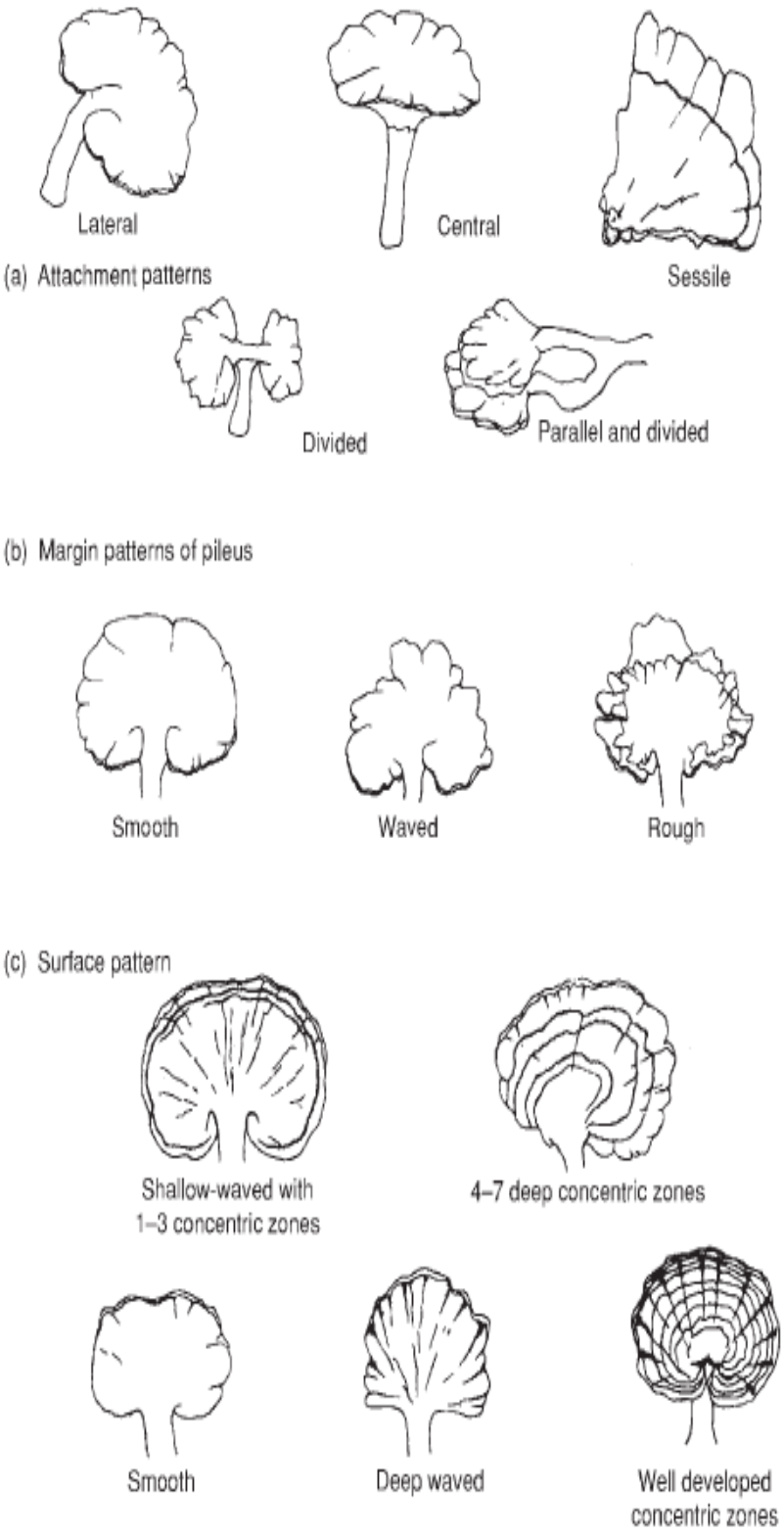


**Figure 4.** Macro-morphological characteristics of the *GL* complex (Flood, Bridge, & Holderness, 2000)

Complex a: attachment pattern, Complex b: Margin patterns of pileus, Complex c: Surface pattern

The taxonomical classification of *GL* is described below (Roberts, 2004; Sanodiya et al., 2009):

Kingdom: Fungi

Division: Basidiomycota

Class: Basidiomycetes

Order: Aphyllophorales (Polyporales)

Family: *Ganodermataceae (Polyporaceae)*

Genus: *Ganoderma*

Species: *Ganoderma lucidum P. Karst*

In previous years, the *GL* taxonomy was usually based on classical descriptive criteria (Chang & Miles, 2004) which results in taxonomic mistakes. Since then, the number of taxonomic names in the genus *Ganoderma* has been increasingly reported with around 290 names (Ryvarden, 2000), contributing greatly to the confusion of the naming of species within this genus. Recently, the taxonomy of the *Ganoderma* species is no longer based on out of date identification methods, including morphological features, physical and developmental characters and chemical components (Roberts, 2004) to avoid such taxonomic errors. Therefore, a wide range of alternative taxonomic approaches are now used, including biochemical tests, sequence analysis of ribosomal genes and spacers and rDNA analysis (Chang & Miles, 2004; Gottlieb, Ferrer, & Wright, 2000; Roberts, 2004). This ensures that the *Ganoderma* species in the genus is well established as well as universally accepted.

*2.2 Bioactive compounds in GL*

There are over 240 secondary compounds which have been found in *GL* (Baby, Johnson, & Govindan, 2015) of which the triterpenoids and polysaccharides are the major constituents and these components also possess significant pharmacological activities (Boh et al., 2007; Chang & Miles, 2004; S. Chen et al., 2012; Paterson, 2006). Specifically, there are more than 150 triterpenes (Boh et al., 2007) and 50 polysaccharides (Jong & Birmingham,

1992) that have been isolated and are known to be unique compounds in this fungus (Chang & Miles, 2004; Wachtel-Galor et al., 2011). In addition, *GL* gives a remarkably strong bitter taste, which has not been found in any other mushroom (Mizuno et al., 1995; Roberts, 2004). Consequently, products derived from *GL* with different contents of triterpenes or polysaccharides or combinations of these two groups give different pharmacological effects (Jong & Birmingham, 1992). Additionally, there are other compounds present in *GL* in smaller amounts, which also have health effects, such as proteins ("LZ 8"), peptides, amino acids, nucleosides, fatty acids and alkaloids (P. Li, Deng, Wei, & Xu, 2013). Table 1 lists the important bioactive components found in *GL* and their biological functions.

**Table 1.** Therapeutic effects and bioactive components of *GL* reported in the literature until 2015.

| Therapeutic effects | Bioactive components | References |
|---|---|---|
| Immunomodulation: mitogenic activity, stimulation of immune effector cells and complement system | Protein LZ-8, ganoderic acid, β- D-glucan | Won, Lin, and Wu (1992); H. W. Kim, Shim, Choi, and Kim (1997); Kohsuke et al. (1991); Mary Haak-Frendscho, Kohsuke Kino, Toshio Sone, and Jardieu (1993); LX Zhang, Mong, and Zhou (1993); S. Y. Wang et al. (1997); H. S. Chen et al. (2004) |
| Anti-cancer, anti-tumour, chemo and radio prevention. | β-D-glucans, heteropolysaccharides, glycoproteins, lanostanoids, 3β-hydroxyl-26-oxo-5α-lanosta-8, 24-dien-11-one, and steroid, ergosta-7, 22-dien-3β, 3α, 9α-triol. | Cheong, Jung, and Park (1999); Kishida, Okuda, Sone, and Misaki (1988); Wasser and Weis (1999); Kao, Jesuthasan, Bishop, Glucina, and Ferguson (2013); Harhaji Trajković et al. (2009); Huan, Yan, and |

| | | |
|---|---|---|
| | | Zhu (2011); P. Y. Wang, Zhu, and Lin (2012); Y. Zhou et al. (2006); Liang et al. (2014) |
| Anti-cancer (colorectal) | Extracts/ unknown compounds | Hong, Dunn, Shen, and Pence (2004) |
| Anti-cancer (cervical, ovarian, endometrial) | Extracts/ unknown compounds | X. P. Chen et al. (2010) |
| Anti-cancer (prostate) | Extracts/ unknown compounds | Noguchi et al. (2008); Sliva et al. (2004) |
| Anti-cancer (liver) | Triterpenes, | Weng, Chau, Chen, Chen, and Yen (2007) |
| Anti-cancer (lung) | Lucialdehydes A-C | Jiang-Jing Gao et al. (2002) |
| Anti-HIV-1, anti-HIV-1-Protease | Triterpenes, Lucidenic acid O, Lucidenic lactone, Ganderiol, Gandodermanontriol and Ganoderic acid | El-Mekkawy et al. (1998); Hobbs (2002); McKenna, Jones, and Hughes (2002); Gao, Zhou, Huang, and Xu (2003); Min (2000) |
| Anti-diabetic activities | Glycans: Ganoderans B and D | Hobbs (2002); McKenna et al. (2002); Mohammed, Adelaiye, Abubakar, and Abdurahman (2007); Ma et al. (2015); Jia et al. (2009) |
| Hepatoprotective activities | Ganoderic acid R, S and Ganosporeric acid A | R. Y. Chen and Yu (1993); Z. B. Lin, Wang, Liu, and Che (2002) |
| Anti-inflammatory | Ganoderic acid C, 3-oxo-5α-lanosta-8, 24-dien-21-oic acid | Joseph (2009); Ko, Hung, Wang, and Lin (2008); J. M. Lin, Lin, Chiu, Yang, and Lee (1993) |
| Anti-allergic effect | Ganoderic acids C and D | Gao and Zhou (2002); Smith, Rowan, and Sullivan (2002) |
| Anti-androgenic effect | Ganoderol B | Fujita et al. (2005); Liu, |

| | | |
|---|---|---|
| | | Shimizu, Konishi, Kumamoto, and Kondo (2007); Jie Liu et al. (2007) |
| Anti-herpetic effect | Acidic protein bound polysaccharides | Liu et al. (2004); Oh, Lee, Kim, Eo, and Han (2000) |
| Anti-oxidant effect | Chloroform extract/water extracts | Joseph (2009), Kan, Chen, Wu, Wu, and Wu (2015); Shi, Zhang, and Yang (2013); Joseph (2009); Kozarski et al. (2011); Smina, Mathew, Janardhanan, and Devasagayam (2011) |
| Anti-microbial activities: anti-viral, anti-bacterial, anti-fungal effect | Neutral protein bound polysaccharide, acidic protein bound polysaccharide, ganodermin | Hobbs (2002); McKenna et al. (2002); Stamets (2000); Gao et al. (2003); Smith et al. (2002); Wadt, Okamoto, Hi, and Bach (2015); Eo, Kim, Lee, and Han (2000); H. Wang and Ng (2006); Ren et al. (2014); Smolskaitė, Venskutonis, and Talou (2015) |
| Estrogenic effect | Ethanol extracts | Shimizu, Liu, Miyamoto, and Kondo (2006) |
| Anti-mutagenic effect | Methanol extract | Lakshmi, Ajith, Jose, and Janardhanan (2006) |
| Anti-ulcerogenic activity | Polysaccharides | Gao, Zhou, Wen, Huang, and Xu (2002) |
| Anti-proliferative activity | Ganoderic acid T | Hong et al. (2004); Sliva et al. (2004); Hu, Ahn, Yang, Lee, and Kang (2002); Tang, Liu, Zhao, Wei, and Zhong |

| | | |
|---|---|---|
| | | (2006) |
| DNA damage | Water-soluble polysaccharides | K. C. Kim and I. Kim (1999) |

Due to bioactive compounds, namely hypoglyceric polysaccharides, bioactive oxygenated triterpenes, immunomodulatory protein Shiao (2003) and laccase (multicopper containing oxidase) (Joo et al., 2007), *GL* has drawn attention from numerous scientists looking for the mechanism of action of those compounds, due to their potential application in cancer treatment and prevention. For example, the *GL* polysaccharides appear to be the potential bio-actives in *GL*'s role in anti-carcinogenic (Harhaji Trajković et al., 2009; Zong, Cao, & Wang, 2012), and anti-tumourigenic activities (J. Huang et al., 2015), anti-oxidant pathways (I. C. Ferreira et al., 2015), and anti-angiogenic and cytotoxic effects (Boh et al., 2007; Xu, Chen, Zhong, Chen, & Wang, 2011) by scavenging free radicals and decreasing cell damage by mutagens (Boh et al., 2007) as well as providing the hypoglycemic activity via accumulation of plasma insulin levels or decreasing plasma sugar levels (Ma et al., 2015). It has also been reported that polysaccharides exert their effects through the immune-modulatory mechanism. The triterpenes, however, display hepatoprotective, anti-hypertensive, hypocholesterolemic and anti-histamic plus anti-tumourigneic, hypoglycemic effects and anti-angiogenic activity (Boh et al., 2007; Chang & Miles, 2004) since they directly suppress growth and invasion behaviour of cancer cells (Dudhgaonkar, Thyagarajan, & Sliva, 2009). These different compound classes will now be discussed.

### 2.2.1 Triterpenes

Triterpenes are a subtype of terpenes and are composed of six isoprene units that produce linear chains or ring-like structures (Bishop et al., 2015). Figure 5 shows the chemical structure of triterpenes based on lanosterol that is reported to play a vital role as an intermediate in the biosynthetic pathway for steroids (Nes, 2011) and triterpenes in microorganisms and animals (Chang & Miles, 2004). Jong and Birmingham (1992) reported that different triterpenes are known to occur in *GL*, of which ganoderic ($C_{30}$) and lucidenic

($C_{27}$) acids are the most prolific compared to ganodermic acids, ganoderenic acids, ganolucidics, applanoxidic acids, lucidones, ganoderals, and ganoderols, which are also present (Figure 6).

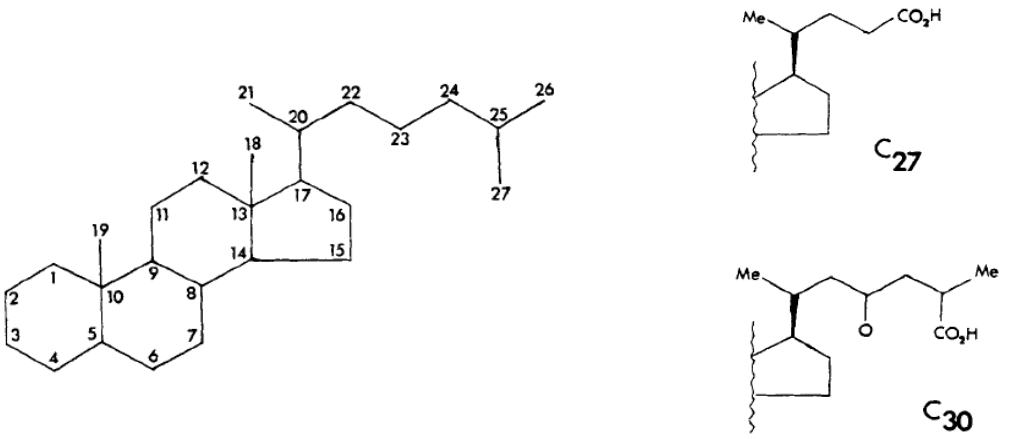


**Figure 5.** Lanostane triterpenoids skeleton (left) (Jong & Birmingham, 1992) and two typical terpenoids of treterpenes (right): $C_{27}$: terpenoid and $C_{30}$: terpenoid.

Ganoderic acids (one type of derivative of triterpenes) can be divided into three groups based on the locations from where they were extracted. For example, ganoderic acids A, B (type I) were detected only in the fruiting body, while ganoderic acids R, S and T (type III) were the major triterpenes of the mycelium (Jong & Birmingham, 1992). Six lanostane-type triterpenes isolated from spores were also determined, such as ganoderic acids ε, ζ, δ, η, θ (Min, 2000). Recently, a greater number of triterpenes in *GL* have been identified and named. There are at least 150 lanostane-type triterpenoids identified and divided into ten groups according to their structural similarities and known biological and medicinal activities (Chang & Miles, 2004; H. W. Kim & B. K. Kim, 1999; Sanodiya et al., 2009).

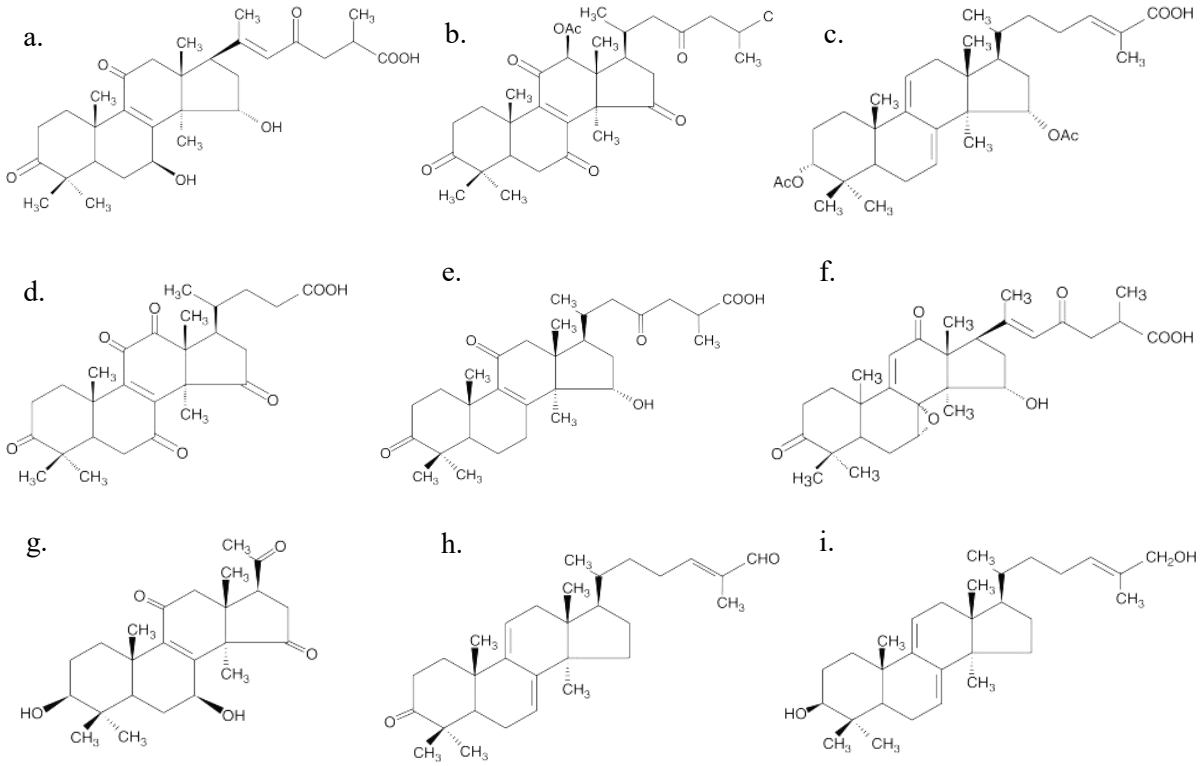


**Figure 6.** Triterpenes with different derivatives

**a:** Ganoderenic acid A ((20 E)-7β, 15α-dihydroxy-3, 11, 23-trioxo-5α-lanosta-8, 20-dien-26-oic acid). **b:** Ganodermic acid F (12β-acetoxy-3, 7, 11, 15, 23-pentaoxo-5α-lanost-8-en-26-oic acid), **c:** Ganodermic acid R ((24E)-3a, 15a-diacetoxy-5a-lanosta-7, 9(11), 24-triene-26- oic acid, **d:** Lucidenic acid D1 (4, 4, 14α-trimethyl-3, 7, 11, 12, 15-pentaoxo-5α-chol-8-en-24-oic acid), **e:** Ganolucidic acid A (15a-hydroxy-3, 11, 23-trioxo-5a-lanost-8-en-26-oic acid) **f:** Applanoxidic acid A ((20E)-15α-hydroxy-7α, 8α-epoxy-3, 11, 23-trioxo-5α-lanosta-9 (11), 20-dien-26-oic acid), **g:** Lucidone (3b, 7b-dihydroxy-4, 4, 14a-trimethyl-11, 15, 20-trioxo-5a-pregn-8-ene), **h:** Ganoderal A ((24E)-3-oxo-5a-lanosta-7, 9 (11), 24-triene-26-al), **i:** Ganoderol B (ganodermadiol-5a-lanosta-7, 9(11), 24-triene-3b, 26-diol) (Boh, Berovic, Zhang, & Zhi-Bin, 2007).

Preliminary studies on triterpenes have reported that they are associated with a bitter taste, which may be derived from various parts of *GL*, but differs from strain to strain of *GL* (Jong & Birmingham, 1992; Wachtel-Galor et al., 2011). In addition, different concentrations of triterpenes are found depending upon the organs of *GL* in which they are formed (spores, fruiting bodies, mycelium, or other parts of *GL*). For example, the spores contain considerably higher contents of triterpenes than other parts of the *GL* (Min, 2000). Moreover, triterpene content increases only after the appearance of the fruiting bodies and these compounds are more concentrated in the outer and older part of the mushroom. Thus, triterpene content varies corresponding to different growth stages of *GL*. Interestingly, it is also reported that

triterpenes varied in bitterness strength depending on the region where the *GL* is produced, including cultivation and climatic conditions (Chang & Miles, 2004).

It has been suggested that the bioactivity of *GL* is related to the level of bitterness derived from triterpenoids (i.e. the more bitter, the greater the bioactivity). However, the relationship between chemical structure of triterpenes and bitterness is not fully understood (Roberts, 2004). Nishitoba, Goto, Sato, and Sakamura (1988) determined that the spatial relationship of hydrophobic methyl groups relative to the three functional oxygen atoms plays an important role in the generation of bitter taste. The authors also determined that the intensity of bitterness was dependent upon different *Ganoderma* species. Triterpenes can be divided into three groups; intensely bitter compounds derived from ganoderic acid A, C1, J, lucidenic acid A, D1, lucidon A, C; slightly bitter compounds such as ganoderic acid B, C2, K and very slightly bitter or no bitter compounds including ganoderic acid D, lucidenic acid B, C, E1, G, ganolucidic acid C, D, lucidon B (Figure 6).

2.2.2 Polysaccharides

Several studies state that fungi produce a variety of high–molecular–weight polysaccharides with different structures (Roberts, 2004; Wachtel-Galor et al., 2011) and they are found in all parts of the mushroom (fruiting bodies, spores, mycelia). It is generally accepted that mushroom polysaccharides have a mode of action that enhances the body's immune response, rather than having any direct cytotoxicity towards tumour cells, which is the accepted mode of action of triterpenes (Dudhgaonkar et al., 2009; Wasser, 2002).

It is reported that the bioactivity of polysaccharides depends on a variety of factors, including; the monosaccharide composition, glycosidic linkage in the main chain structure, and branching and functional groups (Öztürk, Tel-Çayan, Muhammad, Terzioğlu, & Duru, 2015; Lin Zhang et al., 2014). For instance, varying the composition of monosaccharide units within the polysaccharide chain changes their bioactivities. Polysaccharides exhibit their anti-

tumour effects as they mainly contain some specific mono-sugars such as glucose, galactose, arabinose, and ribose (M. Zhang, Cui, Cheung, & Wang, 2007) (Figure 7). Zhang (2007) also reported that polysaccharides, mainly present as certain types of glucans, display immunomodulatory and anticancer activities, dependent on the varied glycosidic linkages in their main-chain structure. For example, glucans that have been demonstrated as the most anti-tumourigenic polysaccharides are those present as β-1,3-D-glucan, β-1,6-D-glucan, and α-1,3-glucan forms (Boh et al., 2007; Ferreira, Passos, Madureira, Vilanova, & Coimbra, 2015; Guo et al., 2009; Zong et al., 2012). Further, a high degree of branching in β-D-glucans (Figure 7) has been positively associated with immuno-stimulatory activities of polysaccharides (Bao, Wang, Dong, Fang, & Li, 2002; Z. Huang et al., 2012; Luo, Sun, Wu, & Yang, 2012; H. Zhang et al., 2012).

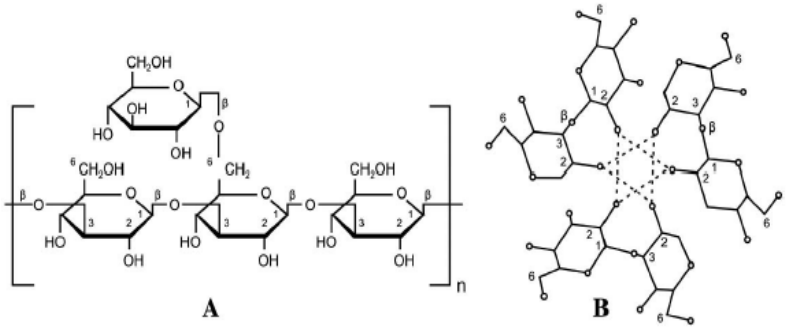


**Figure 7.** Structure of β-D-glucans of *GL*,
A: Primary molecular diagram; B: higher level molecular diagram

*2.3 GL cultivation methods*

Attempts to artificially cultivate *GL* are important for two reasons. Firstly, during the last few decades *GL*-based products have been drawing significant attention from a vast number of people not only from Asian countries but also in North America and Europe (Chang & Miles, 2004). Secondly, it typically takes two to three years for the complete formation of *GL* fruiting bodies in nature, so it is difficult to find natural sustainable supplies of *GL* without artificial cultivation due to its irregular distribution and scarcity (Boh et al., 2007). Therefore, the artificial cultivation of fruiting bodies is known as the fastest and most

sustainable way to produce a sufficient amount of *GL* materials to satisfy the demands of the medicinal herb markets (Boh et al., 2007).

There are several alternative cultivation methods to produce *GL* mycelia and fruiting bodies, namely liquid-state cultivation (LSC) and solid-state cultivation (SSC). The latter can be divided into two methods based on the raw substratum materials used; the log and substituted cultivation methods (Boh et al., 2007; X. W. Zhou, Su, & Zhang, 2012).

2.3.1 *GL* fruiting body cultivation methods

According to Wachtel-Galor et al. (2011), the artificial cultivation of *GL* has become a major source of this mushroom and there is a range of viable substrates which have been applied for cultivation of different *GL* strains: these include grain, sawdust bag and bottle procedures, wood logs (Boh et al., 2007; Wachtel-Galor et al., 2011), short wood segment, wheat straw (Cilerdzic, Vukojevic, Stajic, Stanojkovic, & Glamoclija, 2014), tree stump and cork residues. Although the quality and content of physiologically active compounds (e.g., triterpenes described in Section 2.2.1) varies from strain to strain or from place to place, these traits also depend on culture conditions, the growth stage of fungus, the processing procedures and formulation preparation (Boh et al., 2007). As a result, it is important to find certain cultivation methods which are effective in either increasing the formation of bioactive components in *GL* or the formation of fruiting bodies.

*GL* fruiting body cultivation techniques depend upon the same essential environment factors as in the wild, including temperature, humidity and oxygen (Boh et al., 2007; Cha & Yoo, 1997). These factors are required at different levels in different stages of the artificial cultivation, including the following five stages: spawn run, primordial (antler) formation, primordial (young cork) formation, fruiting body development, and cropping cycle (Roberts, 2004). The entire growth cycle from spawn running to cropping occupies on average approximately 90 to 120 days (Roberts, 2004; X. W. Zhou et al., 2012); however, the time for

artificial cultivation also depends on the method of cultivation used. For example, cultivation on long unsterilised logs, a long traditional incubation method, takes 2-3 years. However, with the new trend of cultivation on short sterilised logs applied in China, Japan, USA and elsewhere, mycelial growth takes only 4 to 5 months and the fruit body can be cropped in the same year (Boh et al., 2007). Interestingly, according to Jo, Cheon, and Ahn (2013), it took only 2-3 months for the mushroom to be ready to harvest when cultivated in sawdust substrates in sterilised bags, called synthetic log cultivation.

In addition to currently published substrate compositions, more researchers are conducting studies using waste products from various industries to cultivate *GL*. This has the additional aim of reducing the environmental load and in the wine industry's case, reducing waste in the form of grape marc. Industry examples include tea waste (Peksen & Yakupoglu, 2008), soy residue (Hsieh & Yang, 2004), stillage grain from rice-spirit distillery (F. Yang, Hsieh, & Chen, 2003) and other residues such as coffee filtrates, straw, sawdust, mixtures of sawdust with rice bran and wheat bran. Interestingly, it appears that there are no studies examining the utilisation of grape marc as a substrate to grow *GL* fruiting body.

2.3.2 *GL* mycelia media

In order to grow *GL* mycelia, both liquid-state fermentation (LSF) and solid-state fermentation (SSF) are popularly used (X. W. Zhou et al., 2012). The former, also known as submerged fermentation, is a process of culturing microorganisms in liquid media, but not on the surface of the media. However, the latter also known as SSC, is the cultivation of microorganisms under controlled conditions in the absence of free water.

In comparison with *GL* fruiting body cultivation, artificial cultivation of *GL* mycelia typically involves five stages: (1) selection of *GL* strains; (2) preparation of culture maintenance medium for different culture phases; (3) inoculation; (4) cultivation of strain in Erlenmeyer flasks, seeding tank, and fermenter, respectively; and (5) harvest (X. W. Zhou et

al., 2012). In addition, in *GL* fruiting body production, the aim is solely to obtain the highest yield of fruiting body mass by weight (kg). However, with *GL* mycelia cultivation, the aim of some studies was simply to produce biomass, with no concern for its composition or to maximise the production of either ganoderic acids or polysaccharides, or to understand how different variables affect their production. Among these studies, many researchers are looking for some substances and specific metabolic products (food medicine, industrial enzymes, etc.) (Chang & Miles, 2004; Patti, Issa, Smernik, & Wilkinson, 2009).

## 3. Research questions

No studies had addressed the following knowledge gaps identified from the literature review:

- Australasian consumers' interests and behaviours towards *GL*-based products, including any novel grape wines made with *GL;*
- Developing a method to cultivate *GL* on a novel substrate using a by-product of the wine industry i.e., grape marc; and
- An examination of the biological compounds of *GL* grown in the grape marc substrate.

Therefore, the study proposed to focus on the following research questions:

- What are the cultural attitudes and emotional factors that determine why Asian consumers would purchase functional foods and beverages containing *GL*, including *GL* wine products?
- What are the chemical composition and sensory characteristics of wine containing *GL*?
- Is it possible to grow *GL* on artificial substrates including grape marc?

The objectives of the study were:

+ To conduct a survey in order to determine the attitudes, consumer behaviour and emotional response of Asian vs Australian consumers toward *GL* functional foods and beverages, including a *GL* wine product.

+ To examine the best methods to produce wine with *GL* which is not only flavoursome but also has health benefits. Furthermore, the production of the new wine provides an opportunity to characterise and compare sensory properties of Australian wine products containing *GL* with conventional wines.

+ To cultivate *GL* with different kinds of cultivation substrates (including grape marc), and compare the yield of biomass of the subsequent *GL* fruiting bodies with those produced using conventional substrates.

## 4. Aims of the project

In the early stages of the project, it became clear that there was a shortage of *GL* mass from cultivation. Given the time frame required to cultivate *GL* material, the main study directions were amended to provide a more detailed focus on the consumer responses to *GL* products, and in particular, *GL* enhanced wine products. The project's aims and objectives towards investigating the sensory and chemical profiles of novel *GL* wine products remained as originally proposed. The literature in respect of the amended study focus, including consumer sensory studies, consumer testing, rapid profiling, and the sensory and chemical characterisation of wines are covered in the introduction/literature review sections of Chapters 2 to 4. This altered research direction leads to the following amended research questions:

- What are the cultural attitudes that determine whether Australian and Asian consumers would accept wines containing *GL* extract?
- How does the presence of *GL* extract in winemaking (Shiraz) impact the primary and secondary fermentations?

- What are the chemical parameters and sensory characteristics of new Australian Shiraz wines containing *GL* extract?
- What is the relationship between chemical composition and sensory attributes in the novel Australian Shiraz *GL* wine products and consumers' liking?

To better understand consumers' attitudes towards *GL* wine products, the study aimed to (1) conduct a survey to determine the consumers' attitudes and response of Chinese and Vietnamese vs Australian consumers towards *GL* functional foods and beverages, including a *GL* wine product (presented in Chapter 2). Once consumers' acceptance and attitudes were determined based on the survey, the next aim of the project was (2) to examine the best methods to produce new wine products containing *GL* and characterise the wine sensory properties as well as chemistry profiles of new wines with *GL* added before or after fermentation (Chapter 3). The study also aimed to (3) investigate the volatile composition and sensorial characteristics of the novel wines that potentially drive consumers' liking with respect to hedonic clusters (Chapter 4).

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# Chapter 2

# A cross-cultural examination of Australian, Chinese and Vietnamese consumers' attitudes towards a new Australian wine product containing *Ganoderma lucidum* extract.

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Review

# A cross-cultural examination of Australian, Chinese and Vietnamese consumers' attitudes towards a new Australian wine product containing *Ganoderma lucidum* extract

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ABSTRACT

*Ganoderma lucidum* (*GL*) is a woody mushroom that has been widely used for many centuries in traditional Chinese medicine. Its bioactive-compounds are believed to promote longevity and prevent diseases in humans. With the close proximity of emerging Asian markets, Australian winemakers are beginning to adopt consumer-centric wine product development as a strategy to generate wines with profiles that meet the specific demands of these consumers. This cross-cultural study recruited 412 wine consumers (Chinese, Vietnamese and Australian) to participate in a survey to understand wine consumers' potential acceptance and self-reported intent towards new wine products produced with *GL* extracts and the relationship of their responses with wine neophobicity across cultures. Findings revealed that all consumer groups accepted the notion that *GL* wine products would be worth tasting and they would try them at social events, with Vietnamese consumers being particularly interested.

Using the wine neophobia scale (WNS), three segments containing wine neophiles ($n$ = 110), neutrals ($n$ = 190) and wine neophobes ($n$ = 112) were identified. The results revealed that Australian and Chinese participants were significantly more wine neophilic, compared to Vietnamese. As expected, neophiles were more prepared to taste and purchase *GL* wine products compared to neophobes across all three countries, although no gender differences were observed. The study provides the wine industry insights about consumers' attitudes towards a new *GL* wine product targeted to Australian and Asian markets that could help develop new niche wine categories and enhance consumers' satisfaction.

## 1. Introduction

Mushrooms are recognised as one of the most popular and essential ingredients in either cuisine or folk medicine (Roupas, Keogh, Noakes, Margetts, & Taylor, 2012). There is a vast variety of edible mushrooms that have been used in human diets for centuries due to their specific and unique aromas and mouthfeel (Cheung, 2010; Moon & Lo, 2014). Others, called medicinal mushrooms, have been practically applied as herbal remedies in traditional medicine in Asian and Western countries owing to their richness of nutrients and bioactive-compounds, including polysaccharides, triterpenes, proteins and steroids, among others (Chang & Miles, 2004; Öztürk, Tel-Çayan, Muhammad, Terzioğlu, & Duru, 2015; Raymon, 1996; Sullivan, Smith, & Rowan, 2006; Wasser, 2002; Yaoita, Kikuchi, & Machida, 2015). There are long-held beliefs about the principal health-enhancing and pharmacological effects of medicinal mushrooms such as the anti-proliferative (Ng et al., 2014), anti-tumour (De Silva, Rapior, Fons, Bahkali, & Hyde, 2012; Zaidman, Yassin, Mahajna, & Wasser, 2005), and anti-oxidant (Adebayo et al., 2018; Siu, Chen, & Wu, 2014) activities. *Ganoderma lucidum* (*GL*), a macro-fungus, is one of the most highly revered curative mushrooms and widely used in China (called Lingzhi), Japan (called Reishi), Vietnam (called Linh chi) and Korea (called Yeongji) (Zhou, Su, & Zhang, 2012).

Recently, several studies have investigated *Ganoderma*-based products in different countries. For example, in Korea, *GL* extracts were used during the bread making process to produce a health product with enhanced texture (Chung, Lee, & Kwon, 2004), and to improve the quality and functionality of traditional Korean Yakju wine by using *GL* during fermentation (Kim, Lee, Lee, Choi, & Lee, 2004). Moreover, *GL* was aseptically added to Pilsner beer in Serbia to create a better perceived body in the beer with added health benefits (Leskosek-Cukalovic et al., 2010). However, little research has examined consumers'

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reactions to *GL*-based products, and given the rapid rise of functional foods (Bigliardi & Galati, 2013; HKTDC-Research, 2018) and wine markets (Australian wine: Production, sales and inventory 2016–17, 2018) in both China and Vietnam and their importance for Australian wine producers, a need existed to conduct cross-cultural research involving Asian and Australian wine consumers. For example, psychographic segmentation based on wine neophobia might be undertaken to examine respondents' reactions to *GL*-based wine products (Johnson, Danner, & Bastian, 2017).

Wine has a lengthy tradition as an alcoholic beverage of choice in Australia. According to the Australian Bureau of Statistics, Australian household expenditure on wine accounted for 26% of the total average amount spent weekly on alcohol (ABS, 2017). Furthermore, Australia has become one of the major wine-exporting countries, with an export value of A$2.6 billion in 2017 (Australian wine: Production, sales and inventory 2016–17, 2018), which has seen this product play a key role in contributing to the growth of the national economy. One challenge the Australian wine industry has faced is increased competition and the perceived lack of product differentiation in both domestic and overseas markets (Colman, 2009; Fogarty, 2006). Changing consumer tastes and the emergence of non-traditional, new wine markets globally such as in Asia, places more pressure on winemakers to both “stand out” on the market shelf and meet consumer needs in order to succeed (Ristic, Johnson, Meiselman, Hoek, & Bastian, 2016). One strategy is to create novel wine products. Based on the Australian and New Zealand Food Standards Code (Standard 2.7.4), a wine with additional ingredients would be categorised as a “wine product”. It is noted that numerous wine products of this type are already available e.g. with fruit, spice or herbal additions (Saltman et al., 2017; Saltman, Johnson, Wilkinson, & Bastian, 2015). Similarly, in alcoholic beverage regulations of China and Vietnam, such wine products could be categorised as flavoured wine or alcoholic beverages, which is defined in the National standard of People's Republic of China (GB 15037-2005) and Vietnamese Government Decree (40/2008/ND-CP dated 17/4/2008 on wine production) and the Vietnamese Ministry Circular (10/2008/TT-BCT dated 25/08/2008).

Before attempting to create new wine products, producers need a deeper knowledge of the perception and consumption behaviour of wine consumers for new wines in the different locales; in other words, a consumer-centric new product development (NPD) approach is required. An objective of wine producers could be to adopt a market orientation and develop and manufacture wines using consumers in the NPD process (Grunert et al., 2008). Following idea generation and screening, the next step in the NPD cycle is to test the concept, which may be undertaken with consumer surveys (Kotler & Keller, 2015). However, understanding target consumers' attitudes to new wine product concepts remains a major challenge, particularly for small to medium enterprises within the Australian wine industry.

Suitable methods are required to assess consumers' attitudes towards different products. As described in previous studies, the food neophobia scale (FNS) was developed as a psychometric tool for assessing the reluctance/avoidance of eating novel foods by consumers (Pliner & Hobden, 1992; Ritchey, Frank, Hursti, & Tuorila, 2003; Schnettler et al., 2013). The FNS concept has since been modified and validated for wine. As reported by Ristic et al. (2016), the wine neophobia scale (WNS) was developed to measure the reluctance or avoidance of wine consumers to buy and try never before experienced and novel wines, and could be an applicable assessment tool when conceptualising new wines. Using the WNS for wine product development in culturally diverse target markets for the first time, the main objectives of this study were to understand the attitude of wine consumers, from different countries and wine neophobia segments, towards novel wine products (i.e., those not familiar to the consumers) containing *GL*. Consumer intelligence in terms of acceptance and willingness to try was also gained. Outcomes could assist and guide the successful development of new wine products targeted at Australian and Asian regions.

## 2. Materials and methods

### 2.1. Consumer samples

Wine consumers ($n$ = 412) were recruited either via online social networks or at a central location (popular food market in the central business district) in Adelaide, South Australia (Chinese $n$ = 107, Vietnamese $n$ = 218, and Australian $n$ = 87). Participants were a convenience sample. Chinese and Vietnamese participants were recruited either in their own countries or in Australia (for those who were at the central location), whereas Australians were entirely recruited face-to-face at the central location. Participants were screened against inclusion criteria, including being at least 18-years-old (legal drinking age in Australia, China and Vietnam), having consumed wine within the past 12 months, and being of either Chinese, Vietnamese or Australian nationality. The three nationality sample groups were included to examine potential cross-cultural differences.

### 2.2. Survey design

The survey, administered via Survey Monkey™, consisted of five sections comprising 1) demographic information, 2) alcohol consumption behaviour, 3) wine neophobia scale, 4) general *GL*-based product consumption, and 5) statements in terms of wine consumers' self-reported intent and acceptance of the concept of a new Australian wine supplemented with *GL* extract. Demographic information questions included age, gender, place of birth and education. The second section asked about the consumers' alcohol consumption frequency and usual price point paid for a bottle of wine, and required participants to rate their level of agreement on items relevant to wine to probe consumers' wine purchase intention using a 9-point Likert-type scale anchored at 1 = strongly disagree, 5 = neither agree nor disagree, and 9 = strongly agree. In section three, participants were asked to answer the 8-item wine neophobia scale (WNS) (Ristic et al., 2016). Section four consisted of questions that determined whether participants had knowledge of and had previously consumed *GL*-based products. The last section asked consumers to rate their agreement with a range of statements regarding their attitudes and self-reported intent towards *GL* wine products on the 9-point scale where 1 = extremely disagree, 3 = moderately disagree, 5 = neither agree nor disagree, 7 = moderately agree, 9 = extremely agree. The survey of consumers' self-reported intent and attitudes was approved by The University of Adelaide Human Research Ethics Committee H-2016-194.

The original questionnaire was generated in English and underwent pilot testing by a small group of staff ($n$ = 4) from the School of Agriculture, Food and Wine at The University of Adelaide with extensive experience in consumer questionnaire development and design, to check for errors, ambiguity, logical flow of questions and length of completion time. The questionnaire was next translated into both Mandarin Chinese and Vietnamese by native Chinese and Vietnamese speakers fluent in English. These were then back-translated into English by different translators in order to ensure the meaning of the original content was maintained and that the three versions were consistent with each another. The revised versions were further pilot tested on a small number ($n$ = 3) of postgraduate students from the School. Revision of the questionnaire occurred if tester feedback indicated there were unclear items or other errors after the pilot tests were conducted. In the preamble to the first *GL* related question, information was provided to all the respondents about *Ganoderma* mushrooms having been used for 2000 years in China as a medicinal mushroom, conventionally as a tea, for example, that could prevent diseases such as cancer, stress and high blood pressure. Participants were also alerted to the presence of *Ganoderma* mushroom products on the market in the forms of capsules, powders, teas, etc.

**Table 1**
Demographic characteristics of Chinese, Vietnamese and Australian respondents.

| Demographics | Total sample ($n$ = 412) | Chinese ($n$ = 107) | Vietnamese ($n$ = 218) | Australian ($n$ = 87) |
|---|---|---|---|---|
| | n | % | % | % |
| Gender | | | | |
| Male | 171 | 43.9 | 38.5 | 46.0 |
| Female | 241 | 56.1 | 61.5 | 54.0 |
| Total | 412 | 100 | 100 | 100 |
| Age | | | | |
| 18–34 | 214 | 58$^{a}$ | 57.3$^{a}$ | 31.0$^{b}$ |
| 35–54 | 154 | 38.3 | 38.1 | 34.5 |
| +55 | 44 | 3.7$^{b}$ | 4.6$^{b}$ | 34.5$^{a}$ |
| Total | 412 | 100 | 100 | 100 |
| Education | | | | |
| Non-tertiary | 94 | 6.5$^{c}$ | 18.8$^{b}$ | 52.9$^{a}$ |
| Bachelor's degree | 182 | 42.1$^{a}$ | 52.8$^{a}$ | 25.3$^{b}$ |
| Post-graduate Degree | 136 | 51.4$^{a}$ | 28.4$^{b}$ | 21.8$^{b}$ |
| Total | 412 | 100 | 100 | 100 |
| Wine consumption frequency | | | | |
| Few times per week | 107 | 33.6$^{b}$ | 8.7$^{c}$ | 59.8$^{a}$ |
| Once per week | 102 | 48.6$^{a}$ | 15.1$^{b}$ | 19.5$^{b}$ |
| Once per two weeks | 52 | 6.5 | 15.6 | 12.6 |
| Once per month | 151 | 11.3$^{b}$ | 60.6$^{a}$ | 8.1$^{b}$ |
| Total | 412 | 100 | 100 | 100 |

Values in the same row not sharing the same superscript are significantly different (significance level at $p < .05$, Chi Square tests with consecutive z-tests). Chi-squared values for each of the demographic data are: gender, $X^2 = 1.76$, df = 2, $p = .413$; age, $X^2 = 68.09$, df = 4, $p < .001$; education, $X^2 = 74.80$, df = 4, $p < .001$; drinking frequency, $X^2 = 176.30$, df = 6, $p < .001$.

### 2.3. Statistical analyses

The data collected by Survey Monkey were analysed by various methods using XLSTAT (Ver. 2017, Addinsoft, New York, USA) and SPSS Statistic 24 (2013, IBM Corp, Armork, NY, USA). One-way analysis of variance (ANOVA) with Tukey HSD post-hoc comparison was used to examine responses to 9-point Likert-type scales. For analyses of categorical and frequency data Chi-square tests with consecutive z-test were applied. All analyses were conducted at a significance level of 5%. Two-way ANOVA was used to investigate the effect of nationality and neophobia status on consumers' self-reported intent towards *GL* wine products. Multivariate analysis of variance (MANOVA) was used to examine the effects of gender and education on consumers' responses to wine purchase decision and consumers' attitudes towards *GL* wine products.

## 3. Results and discussion

### 3.1. Participants

Table 1 shows the demographic information for each nationality. The aggregated wine consumer sample ($n$ = 412) consisted of Chinese ($n$ = 107, 26%), Vietnamese ($n$ = 218, 53%) and Australian ($n$ = 87, 21%) respondents. The Australian group was older compared to the Vietnamese and Chinese groups with no significant difference between the latter two. The proportion of participants within the three educational groups differed markedly between Chinese, Vietnamese and Australian samples. The proportion of Chinese and Vietnamese respondents with a bachelor's degree was significantly higher than the Australian respondents (Table 1). As education and age were distributed differently across the three nationalities (Table 1), age and education level were included in the MANOVA model to analyse consumers' responses to wine purchase decision and consumers' attitudes towards *GL* wine products. Since no significant main effects (age*nationality, F = .85, $p = .689$; age*education, F = .95, $p = .537$; nationality*education, F = .87, $p = .656$; age*education*nationality, F = .95, $p = .570$) of education or age, or interaction between education*nationality or age*nationality, were observed, this suggests that the uneven education and age distribution across countries were unlikely to influence the results. Most Australian participants consumed wine at least once a week which aligns with previous Australian studies (Danner et al., 2016; Danner, Johnson, Ristic, Meiselman, & Bastian, 2017; Johnson & Bastian, 2015; Ristic et al., 2019) (Table 1). Consumption frequency was significantly lower for Vietnamese which parallels a previous study showing that Australians consume wine more frequently than Koreans (Yoo, Saliba, MacDonald, Prenzler, & Ryan, 2013).

### 3.2. Impact of gender, nationality and age on wine purchase decision-making

Respondents were asked to indicate their level of agreement on a 9-point scale towards a series of potential purchase drivers involved in their wine buying decisions. Table 2 shows the factors affecting wine consumers' buying decisions in relation to gender and nationality. No significant differences between age groups were observed (data not shown). Among the responses, females scored significantly higher than males on elements affecting their purchase decision-making such as the authenticity of wine (7.2 vs 6.8, $p = .047$), wine with claimed health benefits (6.1 and 5.4, $p = .011$) and environmentally friendly wine (6.2

**Table 2**
Impact of gender and nationality on purchase decision-making.

| Factors impact on buying decision | Gender | | | | Sig. | Nationality | | | | | | Sig. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Male | | Female | | | Chinese (n = 107) | | Vietnamese (n = 218) | | Australian (n = 87) | | |
| | Mean | Std. error | Mean | Std. error | | Mean | Std. error | Mean | Std. error | Mean | Std. error | |
| Wine brand reputation | 6.3 | 0.2 | 6.6 | 0.1 | 0.120 | 6.2$^{b}$ | 0.2 | 6.8$^{a}$ | 0.1 | 6.2$^{b}$ | 0.2 | **0.015** |
| Authenticity of wine | 6.8$^{b}$ | 0.2 | 7.2$^{a}$ | 0.1 | **0.047** | 7.0$^{a}$ | 0.2 | 7.4$^{a}$ | 0.1 | 6.2$^{b}$ | 0.2 | < **0.001** |
| Grape varieties | 6.6 | 0.2 | 6.7 | 0.1 | 0.681 | 6.7 | 0.2 | 6.6 | 0.1 | 6.8 | 0.2 | 0.594 |
| Claimed health benefits | 5.4$^{b}$ | 0.2 | 6.1$^{a}$ | 0.2 | **0.011** | 4.6$^{b}$ | 0.2 | 7.1$^{a}$ | 0.1 | 4.2$^{b}$ | 0.3 | < **0.001** |
| Price | 6.5 | 0.2 | 6.7 | 0.1 | 0.297 | 6.6$^{ab}$ | 0.2 | 6.8$^{a}$ | 0.1 | 6.0$^{b}$ | 0.2 | **0.011** |
| How the wine is made | 5.9 | 0.2 | 6.2 | 0.2 | 0.183 | 5.8$^{b}$ | 0.2 | 6.6$^{a}$ | 0.1 | 5.1$^{b}$ | 0.2 | < **0.001** |
| Awards or medals | 5.4 | 0.2 | 5.5 | 0.1 | 0.940 | 4.8$^{b}$ | 0.2 | 5.9$^{a}$ | 0.1 | 5.2$^{ab}$ | 0.2 | < **0.001** |
| Environment friendly | 5.7$^{b}$ | 0.2 | 6.2$^{a}$ | 0.2 | **0.047** | 5.3$^{b}$ | 0.2 | 6.6$^{a}$ | 0.1 | 5.4$^{b}$ | 0.3 | < **0.001** |

Data were means where 1 = extremely unimportant, 5 = neither important nor unimportant and 9 = extremely important. Values in the same row not sharing the same superscript are significantly different (significance level at $p < .05$, data analysed by one-way ANOVA, Tukey HSD, post hoc tests). Bolded values indicate significant effects at $p < .05$.

**Table 3**
Impact of nationality on wine consumers' opinions towards statements of *GL* wine products.

| Statements for *GL* wine products | Chinese (n = 107) | | Vietnamese (n = 218) | | Australian (n = 87) | | Sig. |
|---|---|---|---|---|---|---|---|
| | Mean | Std. error | Mean | Std. error | Mean | Std. error | |
| I do not know about the *GL* wine products but I think it is worth trying | 6.2 | 0.2 | 6.3 | 0.2 | 5.7 | 0.3 | 0.148 |
| I would like to go to places where *GL* wine product is served | $5.0^{b}$ | 0.2 | $6.0^{a}$ | 0.1 | $4.9^{b}$ | 0.2 | **< 0.001** |
| I would drink almost any *GL* wine products | $4.8^{ab}$ | 0.2 | $5.1^{a}$ | 0.1 | $4.3^{b}$ | 0.2 | **0.003** |
| At a social gathering, I will try *GL* wine products | 6.7 | 0.1 | 6.2 | 0.1 | 6.5 | 0.2 | 0.116 |
| I am keen on drinking *GL* wine products if the price is reasonable | $6.3^{a}$ | 0.2 | $6.7^{a}$ | 0.1 | $5.4^{b}$ | 0.3 | **< 0.001** |
| I am curious about *GL* wine products and would pay more to have wine containing *GL* | $4.7^{b}$ | 0.2 | $6.0^{a}$ | 0.1 | $4.4^{b}$ | 0.3 | **< 0.001** |

Data are mean values of consumer ratings, where 1 = highly disagree, 5 = neither agree nor disagree and 9 = highly agree. Values in the same row not sharing the same superscript are significantly different (significance level at $p < .05$, data analysed by one-way ANOVA, Tukey HSD, post hoc tests, df = 2). Bolded values indicate significant effects at $p < .05$.

and 5.7, p = .047). These results tend to align with a previous study conducted by Barber (2009) who demonstrated that men used less sources of information in wine buying decisions than women.

In relation to the impact of nationality on consumers' buying intention, factors involving brand, authenticity and price of wine were important and significantly different between the three nationality groups ($p < .01$) (Table 2). Previous studies also found factors such as branding, country of origin and price significantly influence wine purchase decisions (Danner et al., 2017; Liu & McCarthy, 2017; Lockshin, Jarvis, d'Hauteville, & Perrouty, 2006; Xiaoling, Leeva, Charlene, & Jun, 2008); however, none of the studies reported cross-cultural aspects. These new findings in relation to differences in consumers' buying intention between Chinese, Vietnamese and Australian groups provide wine producers with crucial information for creating new products and for promoting and selling their wines to Asian and Australian markets. Table 1 shows that Chinese and Vietnamese respondents have low drinking frequency and agreed that price significantly impacted on their buying decision, which supports Batt and Dean (2000) who reported that price or brand are important factors that influence the selection of wines depending on the drinking frequency of wine consumers, with price being most crucial for those who drink less frequently.

Notably, Vietnamese consumers agreed that factors such as claimed health benefits, production and whether the wine was environmentally friendly were important, with mean ratings that were significantly higher (Table 2) than Chinese and Australian consumers. In addition, all three cultures accepted that grape varieties of wine played a role in their wine purchase decision-making but there was no significant difference across the groups ($p = .594$). Although there are differences in the participants' cultural backgrounds and data collection methods across various studies, the importance of grape varieties in consumers' buying decision have been noted previously (McCutcheon, Bruwer, & Li, 2009). Vietnamese respondents considered wine awards and medals moderately important factors for wine purchase decisions. In contrast, Chinese consumers did not place importance on wine awards and medals, which agrees with an earlier study reported by Yu, Sun, Goodman, Chen, and Ma (2009).

Interestingly, this is the first report highlighting that claimed health benefits of wine are a significant purchase decision driver for Vietnamese consumers (Table 2), whereas the Australian response was very similar to that reported by Johnson and Bastian (2015). The perceptions of health benefits by Chinese and Australian wine consumers have been described in other studies; Chinese wine consumers believed that consuming red wine is beneficial for maintaining a healthy cardiovascular system (Somogyi, Elton, Johnson, Bruwer, & Bastian, 2007) and the perceived health benefits from red wine were rated more highly by Australian wine consumers compared to Korean participants (Yoo et al., 2013). Generally, previous studies have shown that differences in consumer purchase intention are related to cultural backgrounds (Hall, Shaw, & Doole, 1997; Schütte & Ciarlante, 2016), which accords with our results indicating the key factors that impact on consumers' purchase decision differed across the three cultures.

### 3.3. GL-based product consumption and attitudes towards GL wine products

One key aspect of the study was to gauge consumer attitudes towards a *GL* wine product. Collectively, less than half (32.8%) of all the participants had tried any *GL*-based products. With regards to nationality for participants, 48.2% of the Vietnamese, 27.1% of the Chinese and only one Australian respondent (1.1%) had previously used *GL*-based products. These findings are perhaps not surprising, given previous studies found that *GL* has been widely renowned as a medicinal mushroom commonly used in traditional Chinese medicine over the past 2000 years, and has been used as a nutraceutical (in the form of a tea or other blends) or in prescription drugs sold in some Asian countries (Bishop et al., 2015). Despite most *GL*-based products worldwide being advertised as dietary supplements (Lai, Gao, & Zhou, 2004), some *GL*-based food products are available on the market (e.g., coffee, hot chocolate, etc.), but the use of *GL* in Australia has not been well reported.

The potential of *GL* based beverages has been demonstrated with a new beer where consumers accepted the enriched version based on sensory properties (e.g., aroma, taste, body) and overall impression (Leskosek-Cukalovic et al., 2010). Regarding consumers' attitudes and self-reported intent towards *GL* wine products, across the three nationalities, there was positive support for *GL* wine products which can be seen in Table 3. Consumers generally agreed with the notion "I do not know about the *GL* wines but I think it is worth trying" (Chinese, Vietnamese and Australian; mean score (standard error) of 6.2 (0.2), 6.3 (0.2), and 5.7 (0.3), respectively) and "at a social gathering, I will try…" (6.7 (0.1), 6.2 (0.1) and 6.5 (0.2)). Somewhat expectedly, Vietnamese respondents exhibited a stronger acceptance of *GL* wine products; i.e., they agreed that "I would like to go to places where this *GL* wine is served" (6.0 (0.1)) and "I am curious about …and would like to pay more to have …" (6.0 (0.1)). In contrast, Chinese and Australian respondents generally disagreed with these statements and mean values were significantly lower ($p < .001$). Chinese and Vietnamese respondents were significantly more likely to agree with the notion that "I am keen on drinking *GL* wine products if the price is reasonable" (6.3 (0.2) and 6.7 (0.1), respectively) compared to the Australian sample, which was relatively undecided (5.4 (0.3)). These results indicated that wine consumers were generally accepting of the *GL* wine products, but their attitudes were influenced by their cultural backgrounds.

### 3.4. Consumer wine neophobia, nationality and GL wine products self-reported intent

The WNS was used to assess wine neophobia, with wine consumers being asked to respond to eight positively and negatively worded items on the 9-point Likert-type scale. The scale returned a Cronbach's Alpha

**Table 4**
Composition of neophobia segments and their relationship to demographics.

| | Wine-neophobia (%) | | | |
|---|---|---|---|---|
| | Neophiles ($n$ = 110) | Neutrals ($n$ = 190) | Neophobes ($n$ = 112) | Sig. |
| Nationalities | | | | |
| Chinese | 39.1[a] | 26.3[a] | 12.5[b] | < **0.001** |
| Vietnamese | 25.2[c] | 55.3[b] | 75.9[a] | |
| Australian | 35.5[a] | 18.4[b] | 11.6[b] | |
| Gender | | | | |
| Male | 44.5 | 43.7 | 34.8 | 0.240 |
| Female | 55.5 | 56.3 | 65.2 | |
| Age | | | | |
| 18–34 | 50.9 | 48.9 | 58.0 | 0.121 |
| 35–54 | 40.9 | 36.3 | 35.7 | |
| +55 | 8.2 | 14.7 | 6.3 | |
| Education | | | | |
| Non-tertiary | 16.4 | 24.7 | 25.9 | 0.108 |
| Bachelor | 40.9 | 46.3 | 43.8 | |
| Post-graduate | 42.7 | 28.9 | 30.4 | |

Percentage of nationality, gender, age, education groups distributed in each wine neophobia segment. Values in the same row not sharing the same superscript are significantly different at $p < .05$. Chi-square values for each of the demographic data are: nationality, $X^2 = 58.33$, df = 4, $p < .001$; gender, $X^2 = 2.85$, df = 2, $p = .240$; age, $X^2 = 7.28$, df = 4, $p = .121$; education, $X^2 = 7.58$, df = 4, $p = .108$. Different superscripts within a row indicate significant differences between wine neophobia by nationality. Bolded values indicate significant effects at $p < .05$.

value of 0.71, suggesting the scale was reliable, based on a cut-off score of 0.70 being acceptable (Nunnally & Bernstein, 1978). Following the protocol outlined in previous studies (Johnson & Bastian, 2007; Johnson & Bastian, 2015; King, Johnson, Bastian, Osidacz, & Leigh Francis, 2012; Quester & Smart, 1998; Ristic et al., 2016), three segments were identified and labelled wine neophiles ($n$ = 110), wine neutrals ($n$ = 190) and wine neophobes ($n$ = 112) (Table 4).

Table 4 demonstrates that there was a significant relationship between wine neophobia segments and nationality ($p < .001$). Australian and Chinese participants were significantly more wine neophilic, compared to Vietnamese. Evidently, Australians and Chinese had similar (positive) attitudes towards new wine while Vietnamese consumers' self-reported intent was notably more reserved, which might be associated with both their low wine consumption frequency (Table 1) and lack of a wine drinking culture in Vietnam (Do, Patris, & Valentin, 2009; Harding & Robinson, 2015). In contrast, the wine market in China is relatively new but increasing rapidly, particularly in the middle class, as a result of the growth in wine production in that country (Fang, Yang, & Zhang, 2017) and a desire of Chinese consumers, with higher disposable incomes, to be viewed as wealthy and sophisticated (Camillo, 2012), whereas Australia has a longer history of producing and consuming wine (Bo Liu, McCarthy, Chen, Guo, & Song, 2014; Duarte Alonso & Northcote, 2009; Liu & Murphy, 2007). Wine production and consumption in Vietnam, on the other hand, is in its infancy and is limited but wine plays a crucial role as one of the fastest developing consumer markets in the Vietnamese economy (Le, Thi Nguyen, & Van Nguyen, 2013). According to Harding and Robinson (2015), wine consumption in Vietnam was historically introduced through colonisation by the French but the wine drinking trend slowed when the French left Vietnam in the 1950s (Do et al., 2009; Harding & Robinson, 2015). Whether our findings about Vietnamese wine consumers would differ if it were French wine due to the historical link of Vietnamese consumers to French-branded wines would be an interesting future aspect to address. Indeed, more research exploring the drivers of national differences and wine neophobia as well as understanding consumers' attitudes towards new wines in cross-cultural studies seems warranted. When determining the association between WNS and gender, there was no significant difference between male and female respondents ($p = .240$), which is consistent with previous findings in an Australian study (Ristic et al., 2016). Our results are also consistent with a number of food studies, such as those conducted in Finland (Urala & Lähteenmäki, 2007) and America (Meiselman, King, & Gillette, 2010), where it was concluded that there is no evidence of gender effects on consumers food neophobia score.

Table 4 reveals no significant associations between wine neophobia segments and age ($p = .121$) or education ($p = .108$). This contrasts previous results from Ristic et al. (2016) who found that wine neophobia level increased with age and decreased with higher education as well as Meiselman et al. (2010) who found the same effects for food neophobia. So far, relationships between demographics and wine neophobia segment differ from one research study to another. This might be due to factors such as the diversity of participant nationalities which is worth considering in future studies.

No interactions between wine neophobia status and nationality in relation to consumers' self-reported intent towards *GL* wine products were observed ($p > .05$), indicating that the different neophobia segments responded in a similar way across the three nations. Fig. 1 showed that Vietnamese participants were more accepting of *GL* wine products across all three neophobia segments compared to Australians. The Chinese participants generally ranged between the other two nations' responses, although not being significantly different from Australian responses in most cases. As stated previously, Vietnamese and Chinese participants were more familiar with *GL* and *GL*-based products in general which could be a possible explanation why they were more willing to try *GL* wine products irrespective of their neophobia status.

## 4. Conclusion

The use of the mushroom *GL* as an ingredient supplemented in *Ganoderma*-based products has increasingly drawn the attention of health-concerned consumers around the world due to its well-known health benefits from various studies. Increasingly, *Ganoderma*-based products have been created for purposes including health, cosmetics, and nutraceuticals. The current study focused on a survey of Asian (Chinese and Vietnamese) and Australian wine consumers' acceptance of and attitudes towards the use of *GL* extracts used to supplement wines, with outcomes that may help wine producers more specifically understand the target market in terms of new wine product development. Nationality significantly related to wine neophobia, and in particular, most Vietnamese respondents were wine neophobic, whereas the majority of Chinese and Australian respondents were wine neophiles. Use of *Ganoderma*-based products was much more common for Chinese and Vietnamese consumers rather than Australian, but all national groups are notably accepting of the concept of a new Australian red wine containing *GL*. This should provide encouragement to wine producers who wish to explore opportunities for unique wine products in these markets.

## 5. Limitation

The selection of the consumer samples may not represent the general wine consumers across the three nations and results may be viewed as the first approach that informs future studies on consumers' acceptance of new wines, and especially those containing *GL*. Recruitment of larger samples from various cities in China, Vietnam and Australia in future would make the study outcome more robust and may include a survey of consumers' attitudes followed by sensory testing of wines in these target market countries.

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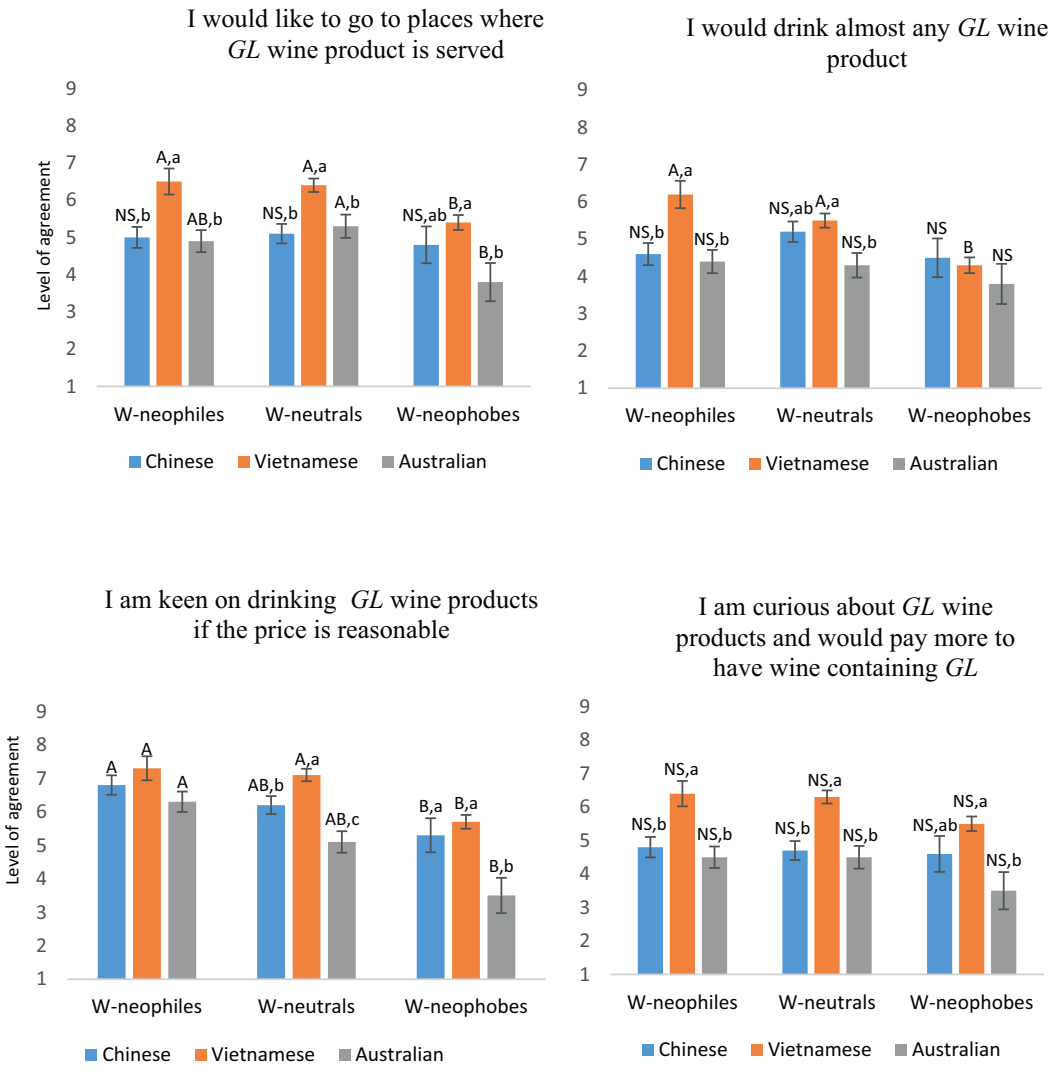


**Fig. 1.** Consumer *GL* wine product attitudes of three nationalities in relation to wine neophobia segments. Error bars indicate standard errors of the mean. Lower case letters indicate significant differences across nationality within a neophobia segment. Upper case letters indicate significant differences between neophobia segments within nationality (Tukey post-hoc comparision, $p < .05$).

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# Chapter 3

# Volatile composition and sensory profiles of a Shiraz wine product made with pre- and post-fermentation additions of *Ganoderma lucidum* extract

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# Statement of Authorship

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| Contribution to the Paper | Created original concept of the study, designed and performed chemical and sensorial experiments: conducted three (small-, medium- and larger-scale) wine fermentations, performed wine packaging and wine presentation in the CATA (n = 11 and n = 32 participants) and RATA sensory panels (n = 65 participants).<br>Conducted preliminary experiment using CATA sensory test to determine *GL* concentrations that were added into a larger-scale wine fermentation. Recruited panellists participated in both CATA and RATA sensory tests via email, social media, local networks and direct invitation.<br>Prepared and conducted basic chemical analyses of 18 wine samples produced by larger-scale fermentation, assisted wine volatile analysis (with HS-SPME-gas chromatography mass spectrometry), collected and interpreted data.<br>Drafted the first draft, edited and revised manuscript. |
| Overall percentage (%) | 70 |
| Certification: | This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper. |
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## Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);

ii. permission is granted for the candidate in include the publication in the thesis; and

iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

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|---|---|
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*Article*

# Volatile Composition and Sensory Profiles of a Shiraz Wine Product Made with Pre- and Post-Fermentation Additions of *Ganoderma lucidum* Extract

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**Abstract:** Novel Shiraz red wine products enriched with *Ganoderma lucidum* (*GL*) extract, a traditional Asian medicinal mushroom, were developed and characterized. *GL* extract was added at different levels prior to and after primary fermentation to investigate its impact on the juice fermentation kinetics, and the chemical composition and sensory properties of the resulting wines. The fermentation kinetics of red grape juice were not significantly different between ferments. Basic chemical analyses plus headspace solid-phase micro-extraction (HS-SPME), gas chromatography–mass spectrometry (GC-MS), and a rate-all-that-apply (RATA) ($n$ = 65) sensory panel were used to investigate the influence of *GL* extract additions on wine composition and sensory characteristics. Of the 54 sensory attributes assessed, 39 significantly differentiated the wines. A clear separation between *GL* wine treatments was evident with PLS regression, where specific volatiles were correlated with relevant sensory attributes that dominated the wines. These products could be promising for emerging wine markets.

**Keywords:** sensory analyses; rate-all-that-apply (RATA); headspace solid-phase micro-extraction (HS-SPME); gas chromatography–mass spectrometry (GC-MS); wine volatiles

## 1. Introduction

*Ganoderma lucidum* (*GL*) is an edible mushroom that has been used in Traditional Chinese Medicine for thousands of years, owing to a belief in its ability to lower cancer risk and the incidence of heart disease, as well as enhance the human immune system [1,2]. In the past, the mushroom was scarce in the wild, and was revered and served as a special food or tea that was believed to prolong the human lifespan due to its nutritional composition [3]. Recently, commercial cultivation has started and *GL* has become readily available on the market [4].

Pharmacological and clinical trials have demonstrated that *GL* can offer a wide range of medicinal benefits [3,5–7]. With the advent of modern science and technology, *GL* has now become a universal biological ingredient found in pharmaceutical powders and capsules [8], dietary supplements [1], and compounded medicines [9]. Previous research has not only reported the positive health effects of *GL's* bioactive compounds such as the triterpene acids and polysaccharides

[10], but also highlighted the successful inclusion of *GL* in a wide variety of foods and beverages [2,4,5]. This explains why *GL* has drawn a large amount of attention from numerous groups working on the research and development of *GL* functional foods and beverages.

Interestingly, several forms of *GL*, including fruiting body powder or extract and mycelia, have been used to produce functional *GL* foods and beverages. *GL* mycelia have been fermented on different substrates for tea production [11] and soy milk fermentation [12]. Additionally, Kim et al. [13] applied *GL* extract during the process of alcoholic fermentation to enhance the functional properties of Korean rice wine (Yakju). Other functional beverages with health-promoting properties have also been produced with added *GL* to improve the perceived body (for example, in a Serbian Pilsner beer [4]) and the sensory properties of grape brandy/distillate wine [14]. Both Leskosek-Cukalovic et al. [4] and Pecić et al. [14] added *GL* extract as a raw material when developing their products: the former aseptically added *GL* extract to commercial Pilsner beer, whereas the latter cut *GL* fruiting bodies into pieces (1 cm) and subsequently mixed them with local homemade grape brandy and wine distillate (40% *v*/*v*). However, neither of these studies investigated whether the presence of *GL* extract impacted the fermentation process, and the kinetics of alcoholic fermentation with *GL* additions have not been well documented.

Although there have been several studies on developing new *GL*-based foods and beverages, there appear to be no reports related to grape wine. Nguyen et al. [15] reported that most consumers in a three-nation study (Australia, China, and Vietnam) had positive attitudes toward *GL* wine products. As such, there is potential demand for these types of products in specific markets such as in Asia, where most consumers are more likely to be familiar with and have a strong belief in the potential health benefits of *GL*. However, a detailed assessment of the chemical composition, including volatiles and sensory attributes such as color, aroma, taste, and flavor of novel *GL*-based products, would need to be undertaken to assess their market potential. To our knowledge, there has been only one study that has examined *GL* extract in an alcoholic beverage fermentation [13]. These researchers demonstrated that the fermentation of a Yakju rice wine with *GL* extract mixed in the rice mash improved the consumer acceptability of the product. However, the volatile chemicals were not measured in this wine, and the sensory analyses were limited. To date, no one has performed an in-depth examination of the sensory profiles and chemical composition of foods or beverages containing *GL*.

This study was conducted to address: (1) the knowledge gap about the impact of *GL* on wine fermentation and (2) the lack of detailed sensory and chemical profiles of foods made with *GL*, with the ultimate aim of exploring the potential of producing a new wine product containing *GL* for the Asian markets. To achieve this, the effect of *GL* addition on the progression and completion of Shiraz red wine primary fermentation was evaluated. Additionally, the differences in chemical composition and sensory profiles between wines made with different levels of *GL* extract added either during or after fermentation were assessed using; basic wine chemical measures, volatile chemical analyses by headspace solid-phase micro-extraction (HS-SPME) coupled with gas chromatography–mass spectrometry (GC-MS), and the rate-all-that-apply (RATA) [16] sensory methodology. Correlations between significant chemical and sensory data were made using partial least squares (PLS) regression to understand the chemical drivers of the perceived sensory attributes identified in the trial wines.

## 2. Materials and Methods

### *2.1. Study Design*

This study consisted of three distinct parts: (1) preliminary experimental small-scale (100 mL) fermentations in chemically defined media and Shiraz grape juice to determine the concentrations of *GL* extract that did not impact on fermentation kinetics and inform the next phase; (2) medium-scale fermentations of Shiraz juice (5 L), which together with commercial wine were evaluated by two preliminary benchtop sensory panels using check-all-that-apply (CATA) to determine the *GL* concentrations suitable for fermentation and sensorial acceptability of wines in (3) where larger-scale winemaking (28 L) was conducted to produce a sufficient number of wine samples that were

subsequently used in a formal descriptive sensory test (RATA) and detailed chemical analyses, allowing for the examination of relationships between the sensory characteristics and chemical components of *GL* wine. Based on the Australian and New Zealand Food Standards (Standard 2.7.4), any such wine would be considered a "wine product," but will be referred to as wine throughout the remainder of the text for simplicity.

### *2.2. GL Extract*

*GL* extract powder (dual alcohol and triple hot water extracted, 1 kg) was purchased from the Super Food Australia Company (Blackheath, New South Wales, Australia) and stored at room temperature (approximately 23 °C).

### *2.3. Fermentation*

*Small-scale fermentations (100 mL)*. Ten grape juice *GL* extract mixtures (100 mL) were produced in triplicate by adding extracts at five different levels (0, 4.5, 9, 18, and 36 g/L) into 100 mL of chemically defined grape juice media (CDGJM) [17] and 100 mL of cross-flow filtered and cold stabilized 2016 Australian Shiraz red grape juice (RGJ) purchased from Patritti Wines (Dover Gardens, Adelaide, SA, Australia). Fermentations were conducted using 250-mL Erlenmeyer flasks fitted with airlocks, as described in a previous study [18]. The strain of *Saccharomyces cerevisiae* (*S. cerevisiae*) used was the commercial wine yeast EC1118 (300 mg/L) (Lallemand, Edwardstown, SA, Australia), which was rehydrated and grown in yeast extract, peptone, and dextrose media (YEPD), consisting of 1% yeast extract (Amyl Media, Dandenong, VIC, Australia), 2% bacteriological peptone (Amyl Media, Dandenong, VIC, Australia), and 2% glucose (Chem-Supply, Gillman, SA, Australia). Fermentation was monitored by measuring the °Brix values of each ferment daily, using a PAL-1 portable refractometer (Atago, Tokyo, Japan) until 6 °Brix between seven and 10 days when ferments had plateaued and were terminated [17]. °Brix values measured in the small-scale fermentation are shown in Table S1.

*Medium-scale fermentations (5 L)*. To determine the acceptable organoleptic levels of *GL* in wine for sensory analyses in the larger-scale fermentations, mixtures of Shiraz grape juice (Patritti Inc., 13-23 Clacton Rd, Dover Gardens 5048, SA, Australia) and *GL* extract were prepared at different levels of *GL* based on the 100 mL experiments. The *GL* concentrations trialed were 0, 4.5, and 9 g/L in 5 L of juice, with the fermentation being conducted using the same protocol as the small-scale fermentations described above.

The resultant three medium-scale wines were assessed by a sensory panel ($n$ = 11 participants, who were either University of Adelaide students enrolled in postgraduate coursework oenology and viticulture programs or higher degree research students aged between 28 and 35 years) using a CATA analysis. Additionally, a second preliminary evaluation of a commercially available South Australian 2016 Shiraz wine (Yalumba, Angaston, Australia; alcohol: 14% *v*/*v*) used as a base wine and enriched with different amounts of *GL* (0, 2.25, 4.5, 6.75, and 9 g/L, added immediately before the benchtop tasting occurred), were examined by a sensory panel ($n$ = 32 University of Adelaide students enrolled in postgraduate coursework oenology and viticulture programs or higher degree research students aged 28 and 35 years). For both CATA panels, 30 mL of each wine were assigned a random three-digit code and presented in transparent ISO-standard glasses, in randomized order for blind tasting for liking, intensity ratings of specific attributes, and CATA analysis. In the tasting session, participants were first asked to rate their wine liking on a nine-point scale (1 = dislike extremely, 5 = neither like nor dislike, 9 = like extremely) and rate the intensity of seven sensory attributes (aroma, sweetness, acidity, hotness, umami, bitterness, and astringency) on a seven-point scale (1 = extremely low, 4 = moderate intensity, 7 = extremely high) for each wine. The last part of the sensory session involved the CATA, where participants only ticked aroma or flavor attributes that they perceived to be present in the wine based on an attribute list (tropical, lychee, citrus, red berry, cherry, dark berry, dried fruit, jammy, confectionery, floral, honey, herbaceous, oak, sweet oak, leather, tobacco, spice, pepper, earthy, mushroom, and savory notes) generated by an expert benchtop trial with five wine academics. Panelists individually rated each wine in an open-plan sensory facility and took a 1-min

forced break between each wine, and had access to water and plain crackers as palate cleansers. Data were collected by paper ballot.

Preliminary CATA analysis of the commercial Shiraz wines with added *GL* (Table S2) showed that the majority of sensory attributes were not significantly different between wines, with the exception of earthy and mushroom aromas, which were noted significantly more in *GL*-treated wines and oak, which was significantly lower in *GL*-treated wines than in the control wines. In the medium-scale fermentation wines, significantly higher floral, tropical, and lychee flavors were found in the control wine (Table S2).

Regarding the intensity of aroma and palate attributes, increasing the levels of *GL* addition (up to 9 g/L) in commercial Shiraz wines did not significantly impact the intensities of acidity, heat, umami, and astringency, but the aroma intensity and bitterness were significantly higher in the 9 g/L *GL* wine (Table S2). Sweetness differences were not as clear, but the 4.5 g/L wine was significantly sweeter than the 6.75 g/L wine. In the case of extracts added during the medium-scale fermentation, *GL* wines were perceived as significantly less sweet, more acidic, hotter, and more bitter than the control wines (Table S2).

*Larger-scale fermentations (28 L).* Juice (500 L) was sourced from Patritti Inc. (2017 Australian Shiraz; 22 °Brix, pH 3.4; 3.9 g/L titratable acidity (TA)). Before fermentation commenced, the juice was dispensed into 28 L batches in sterilized (hot water and 70% ethanol-washed) plastic drums (30 L) and stored frozen at −15 °C until required (the fermentation process flow diagram is shown in Figure S1). The frozen juice was thawed for two days at room temperature, then mixed with different concentrations of *GL* extracts, or fermented and then mixed with *GL* extracts. Treatments conducted in triplicate comprised *GL* added before fermentation at 1 g/L (PRE 1a, b and c), 2 g/L (PRE 2a, b and c), and 4 g/L (PRE 4a, b and c), and after fermentation (at bottling) at 1 g/L (POST 1a, b and c) and 4 g/L (POST 4a, b and c) eventually resulting in a total of 18 wines. Juice treatments were thoroughly mixed to ensure the liquid and extract were fully homogenized before fermentation. The concentrations of *GL* extract added into the juice before and after fermentation were determined by a literature review of other foods and beverages supplemented with *GL* [13] and from the preliminary CATA sensory experiment results (Table S2), which showed that the wines were neither liked nor disliked at added *GL* levels ranging from 0 to 6.75 g/L, while the wines with 9 g/L were not liked and were perceived as significantly hotter and more bitter than the wines with lower levels of *GL* addition. Therefore, the concentrations of *GL* applied in 28 L winemaking were 0, 1, 2, and 4 g/L at different stages of the fermentation process. Control wines (control a, b, and c) were fermented under the same fermentation conditions, but without extract addition. After the addition of 100 mg/L diammonium phosphate (DAP), the juice was inoculated with 300 mg/L of Lalvin EC1118 yeast (Lallemand) and co-inoculated after two days with 100 mg/L of Lalvin VP41 malolactic bacteria—*Oenococcus oeni* (Lallemand). Alcoholic fermentations were performed in a temperature-controlled room at 17 °C. During alcoholic fermentation, the °Brix of each fermenter was monitored daily using a density meter (Anton Paar DMA 35, Graz, Austria) until approximately 2 °Brix. Dryness (i.e., residual sugar (RS) < 2 g/L glucose and fructose) was determined enzymatically with a K-FRUGL test kit (Megazyme, Wicklow, Ireland), internally calibrated using 4 calibrators of 0, 0.75, 1.5, and 3.0 g/L of each sugar (D-(-)-fructose and D-(+)-glucose (Sigma, St. Louis, MO, USA)). Malolactic fermentation (MLF) was considered complete when the malic acid levels were in the range of 0.1–0.4 g/L (L-malic acid enzymatic test kit, Vintessential Laboratories, Dromana, VIC, Australia). After malolactic fermentation, wines were racked off gross lees, 60 mg/L of potassium metabisulfite (PMS) was added as an aqueous solution (10% *w*/*v*), and wines were cold-stabilized at 0 °C for 21 days.

After stabilization, PMS was added to yield free $SO_2$ levels of 40–50 mg/L before bottling. Additions of *GL* extracts to wine after fermentation at either 1 g/L or 4 g/L were made with stirring just before bottling. Wines were bottled by WIC Winemaking Services (The University of Adelaide, Urrbrae, Australia) in 750-mL green Bordeaux-shaped bottles closed with aluminum screw caps (Stelvin caps) under nitrogen gas using a Framax filling system (Serravalle Pistoiese, Pistoia, Italy) and Arol closure system (Costa Enterprises, Canelli, Italy). Bottled wines were stored in a

temperature-controlled room at 15 °C for three months and equilibrated at room temperature (22–23 °C) before sensory analyses and sampling for future chemical analyses.

### *2.4. Basic Chemical Analyses*

Basic juice and wine composition measurements of the larger-scale wines were performed in triplicate, while volatile acidity (VA) measurements were conducted in duplicate. °Brix values were measured in juice using a portable density meter (Anton Paar DMA 35). Free and total $SO_2$ content and VA in juice and wine were determined using the methods described in previous studies [19]. Measurements of pH and TA, color (CIELAB), and ethanol content (% *v*/*v*) were undertaken with a T50 Titrator (Mettler-Toledo, Port Melbourne, Australia), Cintra 4040 (GBC Scientific Equipment, Victoria, Australia), and Alcolyzer ME/DMA 4500 M (Anton Paar), respectively. Yeast assimilable nitrogen was determined enzymatically with a Chemwell 2910 auto-analyzer (following the procedure for K-PANOPA and K-AMIAR kits (Megazyme, Wicklow, Ireland)).

### *2.5. Headspace Solid-Phase Micro-Extraction (HS-SPME-GC-MS)*

For quantitative analyses of the major volatile compounds in the headspace of the larger-scale wines, samples were prepared, extracted, and analyzed according to the method described in a previous study [20]. Analyses were undertaken with a Gerstel MPS auto-sampler (Lasersan Australia Pty, Ltd., Robina, Australia), coupled with an Agilent 7890A gas chromatograph (Agilent, Palo Alto, CA, USA) and combined with an Agilent 5975C mass selective detector (Agilent). Separations were performed with a DB-Waxetr column (60 m, 0.25 mm i.d., 0.25 μm film thickness, Agilent J&W, Folsom, CA, USA) with carrier gas at a constant flow rate of 2 mL/min. All other instrument parameters were as previously specified [20].

### *2.6. Rate-All-That-Apply Sensory Evaluation of GL Wines*

RATA is a rapid sensory method that can use trained panelists or untrained wine consumers to objectively generate sensory profiles of wine, requiring less time and cost than traditional profiling methods such as descriptive analyses (DA). Studies have demonstrated that the RATA sensory profiles generated for multiple sets of wines were comparable to those produced by a DA panel [16] and have been successfully utilized with consumers to profile unfamiliar wines [21].

Regular red wine drinkers ($n$ = 65, aged between 28 and 35 years, 50.7% female) from among postgraduate coursework oenology and viticulture programs and higher-degree research students enrolled at the University of Adelaide were recruited as volunteer panelists to profile the 28 L ferment wines. This study was approved by the Human Research Ethics Committee of the University of Adelaide (Approval No. H-2016-194).

Before the formal RATA sessions, a panel consisting of five wine experts assessed the 18 wines for any faults and decided upon the addition of extra aroma or flavor attribute terms to the generic red wine RATA attribute list described in previous studies [16,22]. Added attribute terms included mushroom, earthy, and tobacco, with the ultimate sensory attribute list consisting of 23 aroma, 21 flavor, and 5 mouthfeel attributes. The sensory panel attended one 40-min formal session per week for two weeks, with nine wines presented at each session. Evaluations were conducted in individual sensory booths at 23 °C. Each wine (30 mL) was presented in transparent ISO-standard wine glasses, labeled with three-digit-codes, and covered with glass Petri dishes at room temperature (23 °C). Wines were served sequentially and monadically in a randomized order, balanced for carryover effects [23]. Panelists assessed the wine samples after smelling (for aroma assessments) and tasting (for flavor assessments), and only rated the intensity of each sensory attribute that they perceived to be present on the line scale, as described in a previous study [16]. A rest of 1-min between samples and a 5-min break after the first four samples was enforced, and water and crackers were provided for palate cleansing.

### *2.7. Data Analyses*

Basic chemical data were analyzed by a one-way analysis of variance (ANOVA) with Tukey's HSD post hoc test using SPSS 23 (IBM Corporation, Armonk, NY, USA). The Cochran's Q test was used to compare the impact of a wide range of *GL* levels on the sensory characteristic of sample wines in the CATA testing. RedJade (Redwood City, CA, USA) online software was used to collect the sensory data generated in the RATA testing. SENPAQ (version 5.01, Qi statistic, Ruscombe, UK) was used to identify the sensory attributes that significantly differentiated the wine samples, using two-way analysis of variance (ANOVA) with participants as random and samples as fixed factors. Fisher's LSD was used for post hoc comparisons. Significant sensory attribute means were subjected to principal component analyses (PCA) using XLSTAT (version 2018, Addinsoft, New York, NY, USA). Volatiles were analyzed by one-way ANOVA using XLSTAT, and all significantly different sensory attribute means and chemical components were subjected to PLS regression analyses along with basic chemical components using The Unscrambler (version 9.7, CAMO software A, Oslo, Norway). All statistical tests were conducted using a significance level of 0.05.

## 3. Results and Discussion

### *3.1. Impact of GL Concentrations on Fermentation Kinetics*

#### 3.1.1. Small-Scale Fermentations

*GL* extract has been shown to have antimicrobial effects [24], so it was necessary to evaluate the impact on yeast by the addition of extracts prior to the fermentation of grape juice by monitoring the changes in sugar content in the must. Initially, small-scale fermentations of either RGJ or CDGJM containing *GL* extract added at different concentrations (0, 4.5, 9, 18, and 36 g/L) were conducted to determine whether the presence of *GL* extract in the juices impacted the kinetics of fermentation. At the beginning of the fermentation, the initial °Brix values were moderately different between the treatments due to the variable extract levels added before fermentation, whereby extracts influenced the refractometer measurements (Table S1). However, the range of means of °Brix value at the beginning on day 0 (25.9 − 23.6 = 2.3 for the RGJ and 24.3 − 21.6 = 2.7 for CDGJM) were similar to those of the samples at the end of fermentation (10.7 − 8.2 = 2.5 for RGJ and 9.8 − 6.6 = 3.2 for CDGJM) (Table S1), which indicated that the fermentation kinetics behavior was similar between the two juices as most of the sugars in each ferment were metabolized in the same time period. However, fermentation was slightly slower for control ferments without *GL* extract between days 2 and 4. Minimal inhibitory concentrations of *GL* extracts recorded in previous studies were 0.0125−1.25 mg/mL [24,25]; therefore, the *GL* levels applied in the small-scale fermentations did not appear to have inhibited the yeast performance in the fermentation process. The presence of a variety of sugars and other metabolites from *GL* extract added at 36 g/L or lower in the juice in the current study did not appear to hamper the wine fermentation in either RGJ or CDGJM (Table S1), but were possibly slightly different to control. It is suggested that further investigations need to be done with high-performance liquid chromatography (HPLC) or enzymatic assays on wines fermented with *GL* to comprehend the residual sugar profile after fermentation, including the polysaccharides noted in previous studies [1,9,26,27]. Future studies could examine the yeast metabolism of *GL* extract to have a better understanding of whether they are able to digest constituents other than sugars originating from *GL* extract.

#### 3.1.2. Larger-Scale Fermentations

Fermentation kinetic behavior of the 28 L wine ferments without (control) and with the presence of *GL* at different levels (1, 2, and 4 g/L) was consistent between samples from the beginning to the end of fermentation (Figure 1). Enzymatic measurements of the residual sugars in these wines were less than or equal to 2 g/L, and therefore the wines were considered to be dry. This indicated that the fermentation process had finished successfully, and the addition of *GL* did not impact on the ability of yeast to undertake alcoholic fermentation. Furthermore, the presence of *GL* extract did not impede the MLF, as all wines contained malic acid levels below 0.4 g/L.

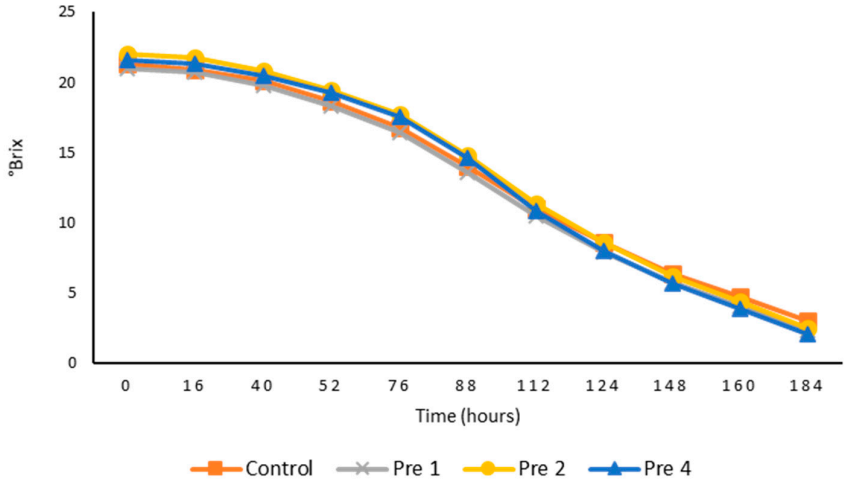


**Figure 1.** Comparative mean °Brix values of triplicate, larger-scale 28 L ferments of control juice and juice with different *GL* additions.

### *3.2. Sensory and Chemistry Profiles of GL Wines*

Compared to the controls, *GL* additions had a significant influence on the perceived red wine sensory attributes. Out of a possible 54 sensory attributes evaluated by the RATA panel, 39 were perceived to be significantly different between the treatments and related to the levels of *GL* addition ($p < 0.05$) (Table S3). The PCA of the mean intensity ratings for statistically significant sensory attributes explained 68.42% of the variation in the data with the first two principal components (PC1 = 39.25% and PC2 = 29.17%, Figure 2). Woody aroma, pepper, and spice flavors; earthy, savory, dried fruit, mushroom, and green capsicum aromas and flavors; astringent and rough mouthfeel were positively loaded on PC1 of the biplot and were associated with wines POST 4a, PRE 2a and 2c, and PRE 4b and 4c. Red appearance, red fruit, confectionery, floral notes, and smooth mouthfeel were clustered positively on PC2, while brown appearance was comparatively strongly negatively loaded and linked to a number of wines including the controls and PRE 1a and POST 1b.

Wines appearing in the top right-hand quadrant possessed green capsicum, spice, and pepper aromas and flavors, mainly associated with wines made with 2 g/L *GL* extract. These wines were also perceived as sweeter in taste that, since the wines were fermented to dryness, may be caused by compounds occurring in the *GL* extract such as polysaccharides [28] and possibly triterpenoids, reported as noncariogenic intense natural sweeteners [29]. The majority of wines in the bottom right-hand quadrant were made with the pre- or post-fermentation addition of 4 g/L *GL* extract and were dominated by mushroom, woody, earthy, toasty, and savory aromas and flavors, higher astringency and roughness, and a bitter taste. Some of these aromas—tobacco, toasty, and woody, for example—are akin to the aromas found in wines aged in oak; it is interesting that *GL* wines were not oaked but could contribute to similar oaked-wine profiles, which might appeal to some consumers [22]. On the contrary, the wines located in the two left-hand quadrants were the controls and those made with pre- and post-fermentation additions of 1 g/L *GL* extract. The wines occurring in the top left-hand quadrant were described as having more red fruit, floral, and confectionery aromas and flavors, a smooth mouthfeel, and a sweet taste. With respect to appearance, wines made with the highest amount of *GL* (4 g/L) were perceived as browner in color, which aligns with the red and yellow tendencies from the CIELAB analyses of these wines. On the other hand, control wines and those with 1 g/L of added extract were more reddish in appearance, which was also evident from the color intensity and blue-green tendencies discussed below (Table 1). In addition, the L* values of wines made with 4 g/L *GL* were lower than those of wines made with 1 and 2 g/L or without *GL* (data not shown), which was supported by a previous study conducted by Pecić et al. [30], who showed that

increasing the levels of *GL* in wines resulted in decreasing L* values. It can be concluded that the wines became slightly darker with an increasing amount of *GL* added.

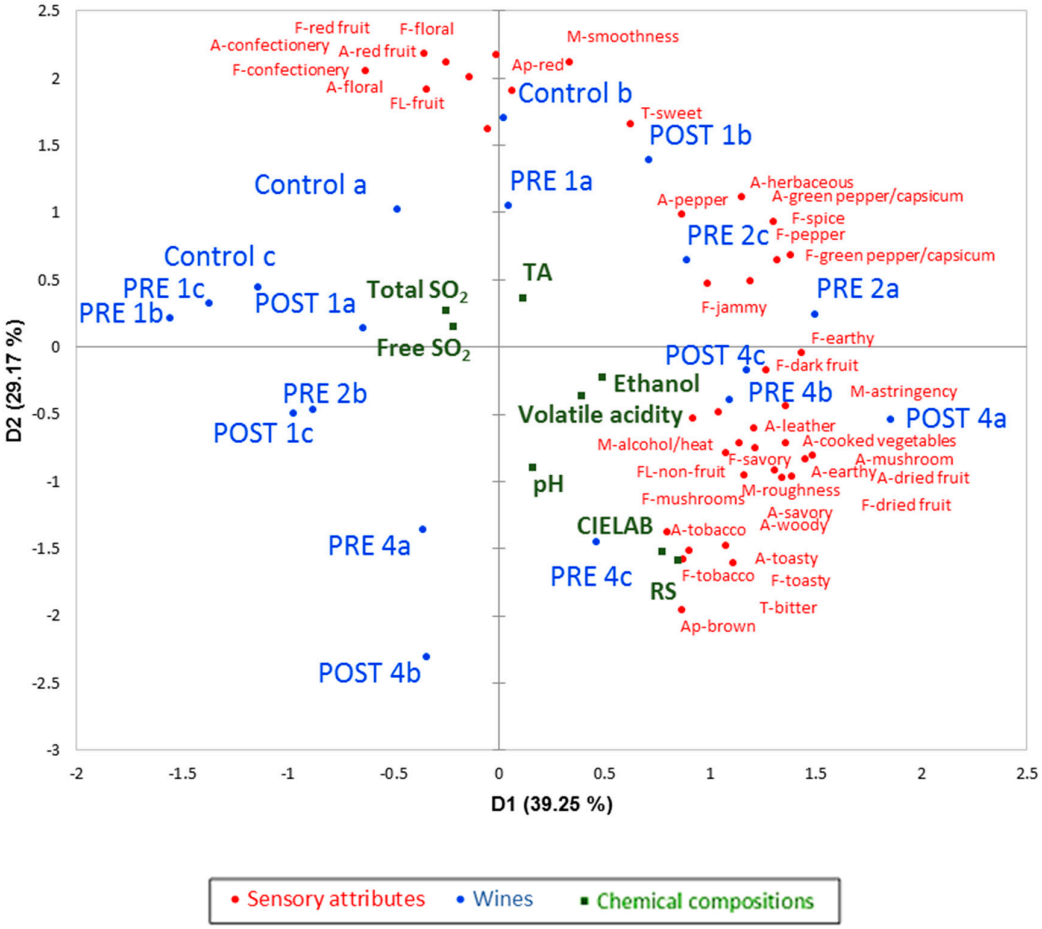


**Figure 2.** PCA biplot of mean sensory data obtained from the RATA panel ($n = 65$) overlaid with basic chemical analyses as supplementary data for 18 wines (28 L ferments) made with and without *GL* extracts added pre- and post-fermentation. Prefix A- = aroma attribute, T- = taste, F- = flavor attribute, M- = mouthfeel, Ap- = appearance, FL- = aftertaste (fruit and nonfruit). Prefixes PRE = *GL* extracts added prior to fermentation (PRE 1, PRE 2, and PRE 4), POST = *GL* extracts added after fermentation process (POST 1 and POST 4).

The current study supports the previous work conducted by Pecić et al. [14], who illustrated that high levels of *GL* added to commercial brandy changed the sensory profile of the resulting products, rendering them more bitter in taste, apparently due to the bitter acids, namely *GL* triterpene acids [3]. In the same way, adding *GL* pre- and post-fermentation in this study generated a variety of wines with new profiles, and the perceived bitterness also increased with increased *GL* additions. From the preliminary benchtop trial of commercial wines spiked with *GL* extract and the medium-scale ferments, a low level of *GL* extract content in wine (2 g/L or less) was preferred and had mean consumer liking scores close to those of the control (Table S2), but 4 g/L or higher was not liked. This may mean that higher *GL* additions promote sensory attributes that sensory panelists may dislike, including the bitterness, roughness, and astringency associated with tannins, and more barnyard aroma, as reported for red wines by Bastian et al. [31] at the expense of the red fruit, floral, and confectionery characters favored by Chinese wine professionals in rosé wines [32]. Comparing Chinese and Australian consumers' preference for red wines, Williamson et al. [33] claimed that consumers from the two countries had similar preference drivers: for instance, red fruit flavor,

sweetness, and a fruity aftertaste were some of the most important sensory attributes positively associated with consumer liking; however, bitterness and strong acidity had low consumer acceptance. Understanding of Australian, Chinese, and Vietnamese consumers' opinions towards *GL* wines was conducted in a previous study and demonstrated their acceptance and willingness to try these wine styles [15]. A preliminary liking study reported by Kim et al. [13] indicated that Korean rice wine with 1 g/L of *GL* extract was the most acceptable compared to rice wines with a higher amount of extract (2 g/L), which caused an unfavorable color and a bitter taste. This is in agreement with our preliminary CATA and hedonic tasting of the medium-scale fermentations (as discussed above), where wines with higher levels of *GL* were less liked, possibly due to the bitterness and astringency being higher in intensity, along with less floral and tropical characters. Therefore, a future study with higher numbers of consumers could be conducted to determine preferences for *GL* wines containing lower levels (e.g., 1–2 g/L) in the Australian and Asian markets.

To produce novel *GL* wines for more extensive sensory and chemical assessment, larger-scale wine ferments with *GL* additions (determined from medium-scale wine sensory analyses) were made. The addition of *GL* pre- (1, 2, and 4 g/L) and post- (1 and 4 g/L) fermentation had a significant impact on the pH, TA, ethanol, VA, and color parameters of the resultant wines (Table 1). The pH values of the wines ranged from 3.8 to 4.0, which are the usual values in accordance with other red wine studies [34,35]. The TA values ranged from 4.1 to 4.7 (g/L), which is slightly lower than the typical range reported in commercial red wines, likely as a result of a lack of skin contact prior to or during the fermentation [36]. As alcohol concentrations ranged from 12.3 to 13.8 (% *v*/*v*) (with a mean value of 13.02% *v*/*v*), these preliminary *GL* wines would be categorized as table wines [37]. The largest difference in % *v*/*v* ethanol between *GL* wines was greater than the recently reported best estimate retronasal and orthonasal difference thresholds measured in a Zinfandel red wine [38]. However, the outcomes from RATA (Figure 2) of the *GL* wines showed that most tasters could only perceive PRE 1b as having a significantly less hot mouthfeel than all other wines.

**Table 1.** Basic chemical composition of the *GL* wines from 28 L ferments.

| Treatment Samples | pH | Titratable Acidity (g/L) | Ethanol (% *v*/*v*) | Volatile Acidity (g/L) | Free $SO_2$ (mg/L) | Total $SO_2$ (mg/L) | Chroma $C^*$(D650) | a* (D650) | b* (D650) | Residual Sugar (g/L) |
|---|---|---|---|---|---|---|---|---|---|---|
| Control a | 3.91 gh | 4.63 b | 12.43 gh | 0.25 f | 48.53 f | 112.53 j | 10.49 m | 8.89 k | 5.58 m | 0.68 hi |
| Control b | 3.91 gh | 4.56 c | 12.75 f | 0.25 f | 48.53 f | 123.73 fg | 10.54 l | 8.85 l | 5.73 l | 0.61 j |
| Control c | 3.93 efg | 4.73 a | 12.79 ef | 0.32 c | 51.20 e | 121.60 h | 10.59 k | 8.81 m | 5.87 j | 0.68 hi |
| PRE 1a | 3.84 i | 4.16 lm | 12.91 e | 0.25 f | 62.40 a | 134.4 a | 11.70 i | 10.11 f | 5.89 j | 0.67 ij |
| PRE 1b | 3.90 h | 4.21 jk | 12.30 hi | 0.25 f | 51.73 de | 117.33 i | 11.67 j | 10.16 e | 5.75 l | 0.74 h |
| PRE 1c | 4.02 a | 4.40 e | 12.73 f | 0.32 c | 52.80 d | 128.53 c | 11.68 i | 10.14 ef | 5.82 k | 0.67 ij |
| PRE 2a | 3.97 b | 4.58 c | 13.32 c | 0.39 a | 48.00 f | 122.13 gh | 11.81 h | 9.80 h | 6.62 h | 0.95 g |
| PRE 2b | 3.93 efg | 4.12 m | 13.36 bc | 0.25 f | 49.06 f | 124.26 ef | 11.82 h | 9.82 gh | 6.59 hi | 1.01 g |
| PRE 2c | 3.92 efgh | 4.29 hi | 13.16 d | 0.25 f | 52.80 d | 125.86 de | 11.82 h | 9.84 g | 6.56 i | 0.98 g |
| PRE 4a | 3.92 fgh | 4.18 kl | 12.25 i | 0.25 f | 56.53 c | 127.46 cd | 12.60 e | 9.55 j | 8.22 c | 1.54 d |
| PRE 4b | 3.93 efg | 4.36 ef | 12.50 g | 0.25 f | 56.53 c | 130.66 b | 12.56 f | 9.59 ij | 8.12 d | 1.68 c |
| PRE 4c | 3.96 bc | 4.48 d | 12.76 f | 0.29 e | 58.66 b | 133.86 a | 12.52 g | 9.62 i | 8.02 e | 1.56 d |
| POST 1a | 3.96 bdc | 4.33 fgh | 13.44 bc | 0.29 e | 40.53 gh | 104.53 k | 12.62 d | 10.35 c | 7.22 f | 1.30 ef |
| POST 1b | 3.96 bcd | 4.25 ij | 13.48 b | 0.31 cd | 40.00 h | 100.80 l | 12.58 e | 10.34 cd | 7.17 g | 1.25 f |
| POST 1c | 3.94 cde | 4.63 b | 13.83 a | 0.34 b | 36.26 i | 90.66 n | 12.59 e | 10.32 d | 7.22 f | 1.32 e |
| POST 4a | 3.97 b | 4.34 fg | 13.48 b | 0.30 de | 36.26 i | 96.53 m | 14.62 b | 11.23 b | 9.37 a | 2.96 a |
| POST 4b | 3.98 b | 4.32 gh | 13.39 bc | 0.31 cd | 41.60 g | 101.86 l | 14.43 c | 11.21 b | 9.08 b | 2.77 b |
| POST 4c | 3.94 def | 4.56 b | 13.45 bc | 0.35 b | 41.60 g | 82.133 o | 14.75 a | 11.62 a | 9.09 b | 2.75 b |

Data are means of triplicate measurements, except for volatile acidity, which was measured in duplicate. Means within a column followed by different letters are significantly different (one-way ANOVA, Tukey's HSD post hoc, $p < 0.05$). The relative standard deviation of the technical replicates was no more than 4% for each wine. a*, b* expressing the green–red and blue–yellow color components, respectively. Prefixes: PRE = *GL* extracts added prior to fermentation, POST = *GL* extracts added after the fermentation process.

Wine color is one of the most important wine quality factors as it impacts sensory assessments and plays a vital role in the decision-making of consumers preferring deeply-colored red wine [39].

The pre- and post-fermentation addition of *GL* extract impacted wine color (Table 1). As a good representation of human color perception, CIELAB measures were used to assess wine color, where L* represents lightness (data not shown), and a* and b* represent the extent of green–red and blue–yellow color, respectively [39]. Chroma C* values ranged from 10.4 to 14.7, with higher color values observed with greater additions of *GL*, which is in agreement with previous studies [13,30]. Our results suggest that the *GL* wines would be noticeably different in color $\Delta E^*_{ab}$ between low (1 g/L, more red appearance) and high (4 g/L, more brown appearance) level treatments ($\Delta E^*_{ab}$ = 2 and 3 CIELAB units for pre- and post-treatment, respectively), which was consistent with the RATA data (Figure 2) that determined wine color significantly differentiated samples. Furthermore, the wines produced with the *GL* extract added prior to fermentation had not only a significantly lower calculated color intensity, but also lower a* and b* values (1 g/L: a* = 10.1, b* = 5.8; 4 g/L: a* = 9.5, b* = 8.1) compared to wines with the same level of *GL* extract added after fermentation (1 g/L: a* = 10.3 and b* = 7.2; 4 g/L: a* = 11.3 and b* = 9.1). The RATA sensory test indicated that the 4 g/L wines were perceived to be deeper in color (L* = 83) than control wines and wines with 1 g/L *GL* addition (L* ranged from 85 to 88, respectively), as the L* value reduced along with an increasing *GL*. These findings confirm that the RATA panel results were consistent with the CIELAB measures, indicating that the panel was performing to a high level. Bisson [40] reported that wines are defined as dry when their RS values are less than 4 g/L at the end of the alcoholic fermentation. The range of RS observed in this study was between 0.61 and 2.96 g/L, consistent with the RS level of dry red wines [37], meaning they would not be perceptibly sweet due to grape-derived glucose and fructose.

To date, only a few studies have examined the volatile compounds from *GL* mycelia by HS-SPME-GC-MS (the most abundant being 1-octen-3-ol, ethanol, hexanal, 1-hexanol, sesquirosefuran, 3-octanol, and 3-octanone) [41] and from *GL* fruiting body (the major occurring compounds being 1-octen-3-ol, 1-octanol, and 3-methyl butanal) using HS-SPME-GC-MS [42]. However, no research has investigated the relationships between the sensory characteristics and chemical components of foods and beverages made with *GL* extract. Therefore, the next step of the study examined the correlations between the chemical composition and sensory profiles of the *GL* wine samples and permitted further examination of the impact of adding *GL* extract either pre- or post-primary alcoholic fermentation.

### *3.3. Volatile Compounds in GL Wine*

Trial wines underwent HS-SPME-GC-MS analyses to evaluate a range of volatiles (Table 2). Among the 29 volatile compounds quantified across the treatments, ethyl and acetate esters were the most abundant. These are fermentation-derived compounds and are known to be responsible for fruity and floral notes in wine [43,44]. Furthermore, eight volatile compounds, including 2-phenylethanol and 1-octanol, ethyl acetate, limonene, and hexanoic, octanoic, decanoic, and 3-methylbutanoic acids, were found to differ significantly between the treatments (bolded significant values in Table 2). Nine odorants occurred in wine samples at concentrations higher than their reported odor detection thresholds, including ethyl butanoate, 1-propanol, 3-methylbutyl acetate, ethyl hexanoate, nonanal, 3-methylbutanoic acid, β-damascenone, hexanoic acid, and octanoic acid. In particular, 3-methylbutyl acetate, which contributes to fruity (banana, pear) characteristics, was present at a concentration of 577 μg/L, 16 times higher than the reported odor detection threshold that was reported in other research regarding the odor detection thresholds of these specific volatiles [20,45,46]. Notably, the concentration of β-damascenone was 24 times above the reported odor threshold, emphasizing its possible important contribution to fruity flavors (apple, rose, honey, candy, and citrus) in these Shiraz wine products, which is in agreement with other studies [47,48].

**Table 2.** Concentration of volatile compounds (μg/L) in control and red wines containing *GL* added pre- or post-fermentation in 28 L ferments.

| Compound | Control | PRE 1 | PRE 2 | PRE 4 | POST 1 | POST 4 | Sig | Aroma Detection Threshold (μg/L) |
|---|---|---|---|---|---|---|---|---|
| ethyl acetate | 3793.6 ± 2330.8b | 7018.5 ± 604.1ab | 8252.6 ± 1258.8a | 7755.0 ± 998.4a | 7374.0 ± 1810.3ab | 8016.4 ± 561.9a | **0.021** | 15,000 ** a |
| ethyl butanoate | 26.1 ± 4.6 | 23.9 ± 2.2 | 25.9 ± 2.1 | 26.3 ± 1.3 | 29.4 ± 2.2 | 27.9 ± 2.8 | 0.292 | 20 ** a |
| ethyl-2-methylbutanoate | 0.5 ± 0.0 | 0.5 ± 0.0 | 0.5 ± 0.0 | 0.5 ± 0.1 | 0.6 ± 0.1 | 0.5 ± 0.0 | 0.572 | 1 ** a |
| ethyl 3-methylbutanoate | 0.6 ± 0.1 | 0.6 ± 0.1 | 0.7 ± 0.1 | 0.7 ± 0.1 | 0.7 ± 0.1 | 0.7 ± 0.0 | 0.653 | 3 ** a |
| 3-methylbutyl acetate | 614.1 ± 28.3 | 609.5 ± 124.1 | 636.6 ± 37.5 | 502.2 ± 6.9 | 563.3 ± 69.0 | 539.0 ± 41.9 | 0.156 | 30 ** a |
| ethyl hexanoate | 60.3 ± 3.2 | 65.6 ± 3.2 | 60.8 ± 2.0 | 58.0 ± 6.3 | 66.2 ± 10.4 | 58.0 ± 5.6 | 0.381 | 14 ** b |
| hexyl acetate | 32.3 ± 2.4 | 30.8 ± 5.6 | 29.0 ± 6.3 | 26.5 ± 3.6 | 29.9 ± 7.4 | 25.7 ± 2.7 | 0.603 | 670 ** a |
| ethyl lactate | 5186.6 ± 494.4 | 5165.3 ± 551.4 | 5637.1 ± 565.7 | 5458.7 ± 1000.1 | 5742.9 ± 1164.8 | 5776.8 ± 366.6 | 0.845 | 146,000 ** a |
| ethyl octanoate | 15.7 ± 0.9 | 15.8 ± 1.0 | 16.8 ± 1.6 | 18.0 ± 0.2 | 16.5 ± 1.8 | 15.8 ± 1.2 | 0.235 | 20 ** b |
| ethyl decanoate | 28.5 ± 4.5 | 33.2 ± 3.9 | 41.2 ± 10.0 | 46.8 ± 6.2 | 35.3 ± 10.9 | 31.8 ± 6.2 | 0.093 | 200 ** b |
| diethyl succinate | 1.4 ± 0.0 | 1.6 ± 0.0 | 12.2 ± 1.9 | 19.0 ± 13.6 | 10.8 ± 7.4 | 11.0 ± 3.3 | 0.052 | 1,250,000 ** a |
| 2-phenylethyl acetate | 21.2 ± 1.6 | 21.2 ± 5.1 | 20.8 ± 4.3 | 20.8 ± 3.2 | 16.4 ± 1.7 | 17.0 ± 1.5 | 0.277 | 250 ** a |
| 1-propanol | 9029.4 ± 732.8 | 8357.8 ± 570.6 | 7802.2 ± 770.4 | 8692.7 ± 616.2 | 9394.5 ± 277.2 | 9215.7 ± 589.7 | 0.066 | 500 ** b |
| 2-methyl-1-propanol | 2362.3 ± 161.8 | 2258.3 ± 231.4 | 2190.4 ± 24.5 | 2102.6 ± 46.6 | 2083.8 ± 57.4 | 2136.8 ± 30.3 | 0.107 | 40,000 ** b |
| 1-butanol | 64.3 ± 10.5 | 60.3 ± 11.2 | 67.3 ± 9.6 | 50.8 ± 8.5 | 71.5 ± 8.1 | 61.1 ± 13.3 | 0.29 | 150,000 * a |
| 3-methyl-1-butanol | 17185.2 ± 765.4 | 16411.5 ± 351.2 | 17774.1 ± 515.1 | 16513.6 ± 2882.3 | 17612.4 ± 256.5 | 16733.9 ± 560.0 | 0.69 | 30,000 ** a |
| 1-hexanol | 199.5 ± 13.8 | 194.7 ± 24.7 | 199.8 ± 14.9 | 193.7 ± 14.6 | 209.7 ± 7.0 | 211.1 ± 4.3 | 0.598 | 8000 ** a |
| Linalool | 6.2 ± 0.6 | 6.7 ± 0.7 | 6.7 ± 0.3 | 7.0 ± 1.2 | 7.6 ± 0.9 | 6.8 ± 0.4 | 0.427 | 15 ** a |
| 1-octanol | 2.4 b ± 0.1 | 2.4 ± 0.2ab | 2.6 ± 0.1ab | 2.8 ± 0.2a | 2.6 ± 0.0ab | 2.6 ± 0.1ab | **0.041** | 0.7 ** a |
| α-terpineol | 5.0 ± 0.4 | 5.2 ± 0.1 | 5.6 ± 0.8 | 6.1 ± 0.9 | 5.4 ± 0.3 | 5.1 ± 0.5 | 0.267 | 250 ** b |
| benzyl alcohol | 178.3 ± 18.7 | 179.5 ± 23.3 | 173.8 ± 22.7 | 166.1 ± 18.4 | 178.2 ± 11.7 | 159.9 ± 8.9 | 0.715 | 200,000 *** a |
| 2-phenylethanol | 1443.4 ± 123.0b | 1587 ± 180.3ab | 1545.8 ± 250.5b | 1985.5 ± 162.7a | 1356.9 ± 21.6b | 1354.5 ± 26.4b | **0.002** | 14,000 ** b |
| Limonene | 0.4 ± 0.0b | 0.5 ± 0.0a | 0.6 ± 0.0a | 0.6 ± 0.0a | 0.6 ± 0.1a | 0.6 ± 0.1a | **0.034** | 15 ** a |
| Nonanal | 2.8 ± 0.0 | 2.8 ± 0.0 | 2.8 ± 0.1 | 2.9 ± 0.1 | 2.9 ± 0.1 | 2.8 ± 0.1 | 0.461 | 2.5 ** a |
| 3-methylbutanoic acid | 45.6 ± 0.6bc | 44.1 ± 2.2c | 47.3 ± 0.7bc | 50.7 ± 1.1a | 47.5 ± 0.7ab | 47.4 ± 1.2bc | **0.001** | 33 * a |
| hexanoic acid | 702.9 ± 29.9c | 788.8 ± 13.1b | 849.5 ± 46.4ab | 887.5 ± 12.0a | 779.1 ± 43.6bc | 848.0 ± 4.8ab | **<0.001** | 420 ** b |
| octanoic acid | 1448.5 ± 87.3a | 1392.1 ± 185.5a | 1301.6 ± 112.0ab | 1240.0 ± 67.6ab | 1072.4 ± 71.6b | 1181.0 ± 48.5ab | **0.009** | 500 ** b |
| decanoic acid | 375.7 ± 33.1a | 362.5 ± 13.2a | 330.7 ± 28.0abc | 341.0 ± 13.1ab | 293.3 ± 24.7bc | 271.4 ± 26.2c | **0.001** | 1000 ** b |
| β-damascenone | 24.1 ± 7.4 | 20.7 ± 6.2 | 28.7 ± 2.9 | 21.8 ± 5.2 | 22.9 ± 4.0 | 25.7 ± 6.3 | 0.561 | 0.05 ** a |

Mean values ± standard deviation of the three fermentation replicates. Bolded *p*-values indicate significant differences based on one-way ANOVA. Lower-case letters indicate significant differences between samples based on LSD post hoc comparison, $p < 0.05$). Prefixes: PRE = *GL* extracts added prior to fermentation, POST = *GL* extracts added after fermentation process. * Refers to Mayr et al. [47], ** Refers to Wang et al. [20], *** Refers to Zhao et al. [48]. Thresholds were reported for aqueous ethanol (a) and wine matrix (b).

### *3.4. Correlation between Chemical and Sensory Data of GL Wines*

To explore the underlying relationships between wine chemistry and sensory data, significantly different data for volatile compounds, basic chemical components, and RATA sensory attributes were subjected to PLS (Figure 3). Figure 3A shows that there was a relatively clear separation between wine treatment groups from the PLS scores plot, where the first two factors explained 61% of the variation in wine chemical composition (x-variables) and 37% of the variation in sensory attributes (y-variables). Wines made with *GL* additions before fermentation (PRE) were primarily located in the two top left and right quadrants of the plot (transitioning from lower to higher additions rates, going from left to right), whereas wines made without *GL* (control) and with *GL* supplemented post-fermentation (POST) appeared in the bottom left and right quadrants, respectively (Figure 3A). In

Figure 3B, the first factor (x explained 37% of total variance in chemical composition, y explained 30% of total variance in sensory characteristics) distinguished wine samples on the left side of the plot mainly according to red appearance, red fruit, and confectionery notes, compared to the right side of the plot, which contained brown appearance; spicy, jammy, earthy, and dark fruit flavors; rough, hot mouthfeel; and bitter taste. The second factor (x explained 24% of the total variance in chemical composition, y explained 7% of the total variance in sensory characteristics) separated samples vertically, from the bottom section with a sweet taste to the top section, which was mainly dominated by an herbaceous aroma and green capsicum flavor.

Wine aroma attributes located in the left quadrants, such as red fruit, confectionery, and floral characteristics, were positively correlated with volatile acids (decanoic acid and octanoic acid) and negatively correlated with 1-octanol, 3-methylbutanoic and hexanoic acids, limonene, and ethyl acetate (Figure S2A,B). The study findings were supported by a previous study conducted by Vilanova et al. [44], who determined that decanoic acid and octanoic acid are typically associated with fruit attributes (ripe fruity attributes).

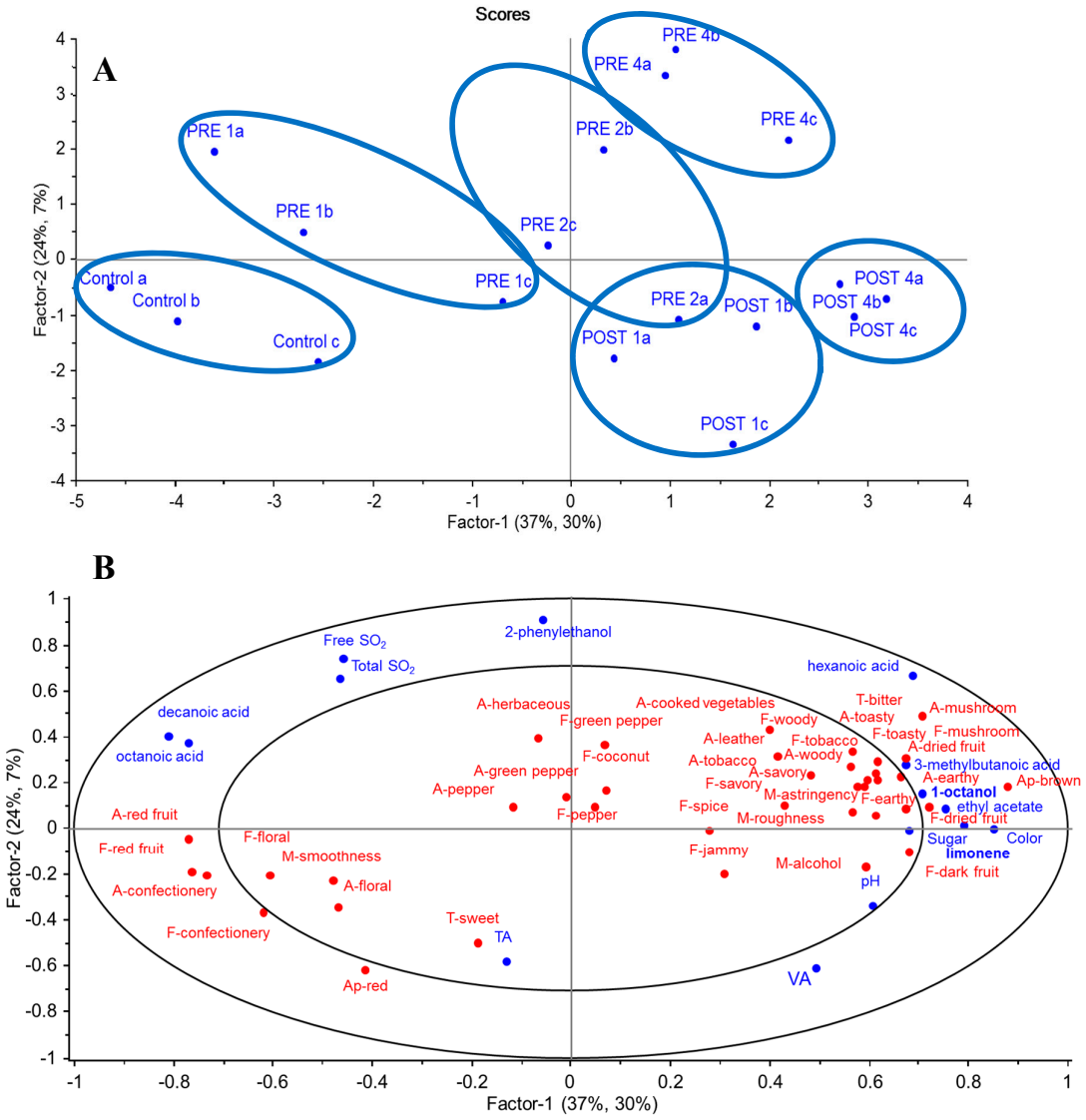


**Figure 3.** PLS regression and scores plots of significant volatile compounds ($p < 0.05$), sensory attributes ($p < 0.05$) and basic chemical data for 18 wines (28 L ferments) made with and without *GL* extracts added pre- or post-fermentation. X-variables: chemical components, Y-variables: sensory descriptors. The inner and outer ellipses represent $R^2$ = 50% and 100%, respectively. (**A**) sample

configuration, prefix PRE = *GL* extracts added prior to fermentation (PRE 1, PRE 2 and PRE 4) and POST = *GL* extracts added after the fermentation process (POST 1 and POST 4). (**B**) attribute configuration with prefix A- = aroma attribute; T- = taste, F- = flavor attribute, M- = mouthfeel, Ap- = appearance, FL- = aftertaste (fruit and nonfruit). TA = titratable acidity, VA = volatile acidity.

Sensory attributes on the right quadrant such as woody, dried fruit, earthy, and mushroom notes and bitter taste were positively correlated with hexanoic acid, 1-octanol, and limonene. Furthermore, hexanoic acid is known for its leafy, wood descriptors [20], while limonene, derived from grapes, relates to floral and citrus (lemon and orange) characteristics in wines [47,49]. Interestingly, Robinson et al. [50], who used PLS regression to predict the relationship between sensory attributes and chemical composition, indicated that both 1-octanol and limonene were correlated with the bitter taste of Cabernet Sauvignon red wines, which aligns with our study findings. In Figure 3, it is noteworthy that a positive correlation was found between 1-octanol and dried fruit (Figure S2C,D), in agreement with a previous study [50]. Similarly, there was a positive correlation between 1-octanol and mushroom notes (Figure S2E,F). Taskin et al. [42] determined that there were 18 aroma compounds found in *GL*'s mycelia including 1-octanol, which implies the 1-octanol in our *GL* wines (4 g/L) could be derived from *GL* extract.

Pre-fermentation *GL* wines appearing in the top section displayed peppery, coconut, green capsicum, and herbaceous attributes, showing a positive correlation with 2-phenylethanol, which imparts floral and rose attributes [47]. On the contrary, wines with *GL* added after fermentation were mainly located in the bottom section, representing less cooked vegetable aroma, a mushroom or leather aroma, green capsicum, toasty, and tobacco flavor, and a less bitter taste.

Considering the potential for producing a *GL*-based wine product [15], various studies were undertaken to investigate aspects of fermentation, chemical composition, and sensory profiles. *GL* extract addition to juice or chemically defined grape juice media was deemed not to overly affect fermentation, with the pattern of sugar consumption being very similar for all the treatments. On the other hand, *GL* extract addition does impact on the sensory and chemical components of the resultant wines. It can be concluded that the higher the concentration of *GL* used in treatments, the more bitter the wines tasted due to the bitterness derived from the triterpenes in the *GL* extract added [30]. Of note, limonene and 1-octanol volatile compounds significantly differentiated between wines and were positively related to wines' bitterness and dried fruit flavor (Figure 3), in accordance with a previous study [50]. It may be possible to remove these bitter compounds with the use of fining agents commonly used in the wine industry, or at least to suppress the bitter taste with the retention of low amounts of residual sugar by not fermenting wines to dryness. The aromatic profile also changed with the highest *GL* addition, with these wines being more complex and having more dark and dried fruit, toasty, earthy, and woody notes. Thus, it is likely that a couple of wine styles could be produced that may potentially suit different consumer segments' preferences.

## 4. Conclusions

This work is the first to report on the impact of *GL* on the primary alcoholic and secondary malolactic fermentation of Shiraz grape wine. Based on our earlier findings that the concept of a *GL*-containing wine was acceptable to consumers from three different countries, we have employed a consumer-centric product development approach to produce prototype wines. Furthermore, we utilized novel and rapid wine sensory evaluation methods and compositional analyses to advance our knowledge of the sensory and chemical properties of Australian-made *GL*-containing Shiraz wines.

*GL* extract addition did not impact wine fermentation, but influenced the sensory profiles and chemical composition of the resulting wines. Fermentation kinetic behaviors were similar between treatments, and stuck or sluggish fermentations were not observed. Thirty-nine sensory attributes, together with eight volatile compounds, significantly differentiated the wine treatments. In addition, specific volatiles correlated with relevant sensory attributes, particularly in 4 g/L *GL* wines. For instance, 1-octanol was positively related to wines' mushroom notes. These initial experiments on

winemaking with *GL* extracts are promising and will enable winemakers to gain insight into potential new wine products, which could be of interest to Asian consumers who display strong consumer behavior in the use of *GL* and its products. Future research could focus on target market consumers' preferences in conjunction with hedonic clustering to determine the specific sensory attributes and chemical components that may drive consumer segment liking. This will assist the wine industry in gaining a deeper understanding of consumers' preferences for novel wines containing traditional Asian medicinal mushroom extracts designed for the Australasian market.

**Supplementary Materials:** The following are available online at www.mdpi.com, Figure S1: Shiraz winemaking process under specific conditions in the 28 L winemaking experiment, Figure S2: Weighted regression coefficients of both aroma and flavor of red fruit (A and B), dried fruit (C and D) and mushrooms (E and F) notes, Table S1: Brix values (°) measured by refractometer from the beginning to the end of small-scale fermentation of chemically defined grape juice media (CDGJM) and red grape juice (RGJ), Table S2: Impact of *GL* on liking, perceived sensory attribute overall intensity and CATA ratings from benchtop evaluations of commercial Shiraz wine spiked with *GL* (panel n = 32) and medium-scale pre-fermentation *GL* wines (panel n = 11) and Table S3: Significant sensory attribute intensity means of *GL* wines (from 28 L ferments) generated by RATA panel.

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# SUPPLEMENTARY INFORMATION FOR

## Volatile composition and sensory profiles of a Shiraz wine product made with pre- and post-fermentation additions of *Ganoderma lucidum* extract

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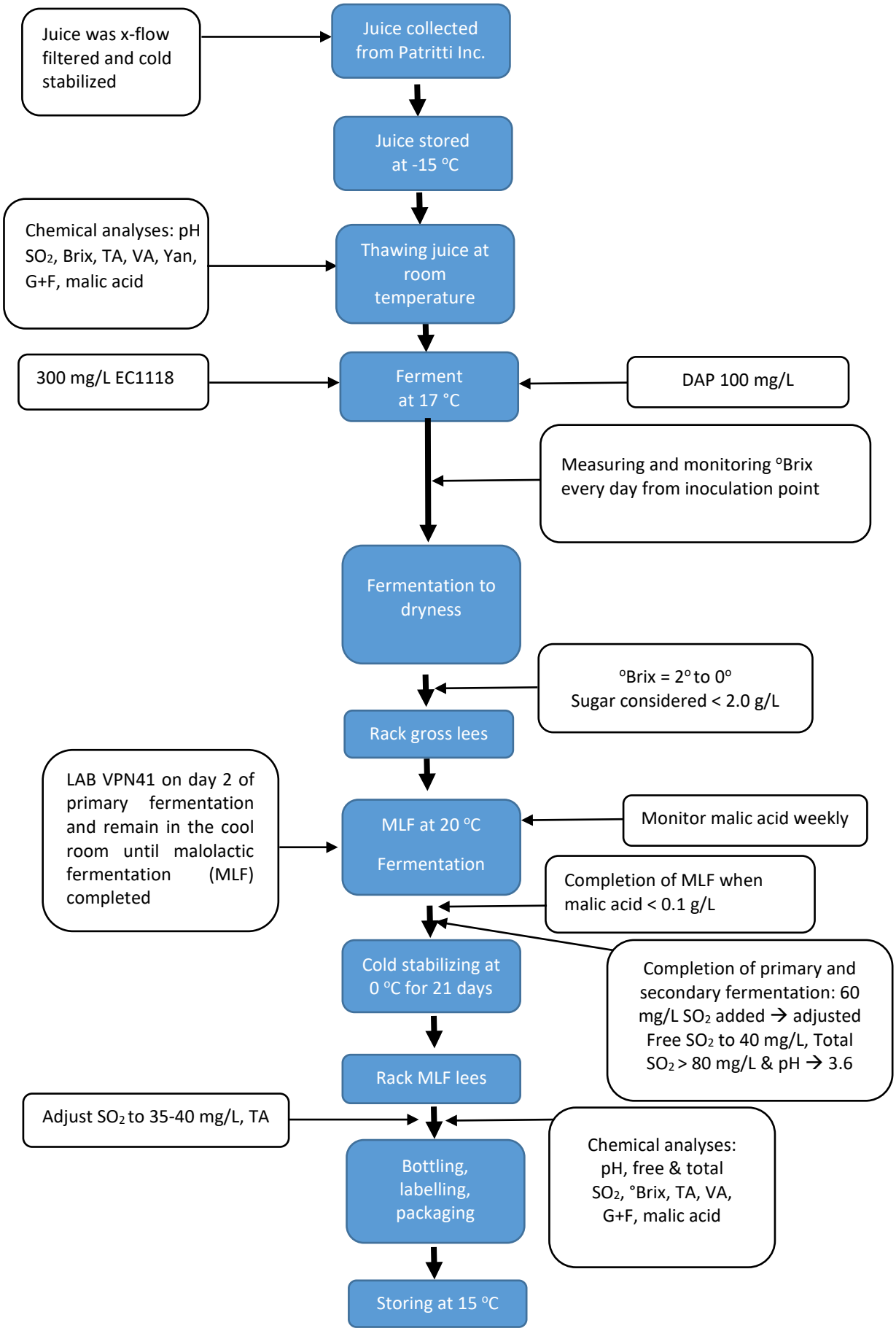


**Figure S1.** Shiraz winemaking process under specific conditions in the 28 L winemaking experiment.

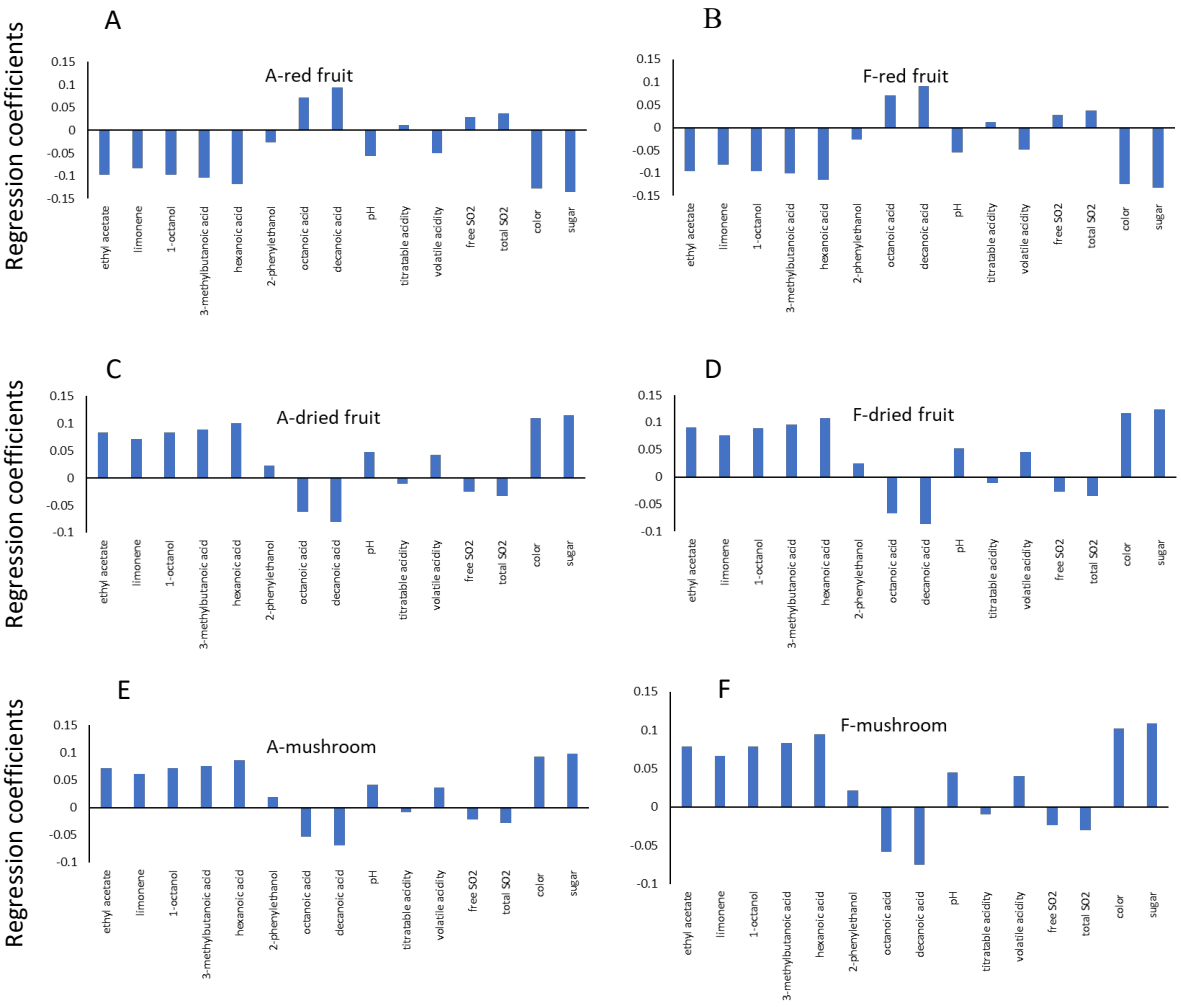


**Figure S2.** Weighted regression coefficients of both aroma and flavor of red fruit (A and B), dried fruit (C and D) and mushrooms (E and F) notes.

**Table S1.** Brix values (°) measured by refractometer from the beginning to the end of small-scale fermentation of Chemically Defined Grape Juice Media (CDGJM) and Red Grape Juice (RGJ).

| | Day 0 | Day 1 | Day 2 | Day 3 | Day 4 | Day 5 | Day 6 | Day 7 | Day 8 | Day 9 |
|---|---|---|---|---|---|---|---|---|---|---|
| CDGJM-0 | 21.6 j | 19.0 d | 15.2 b | 11.9 b | 9.5 d | 8.4 e | 7.2 g | 6.9 h | 6.5 i | 6.6 i |
| RGJ-0 | 23.6 f | 19.9 b | 15.9 a | 13.0 a | 10.8 b | 9.6 c | 8.3 e | 8.4 e | 8.3 f | 8.2 ef |
| CDGJM-4.5 | 22.1 i | 17.3 f | 10.9 g | 7.0 h | 7.2 i | 7.1 g | 7.0 g | 7.2 g | 7.1 h | 7.1 h |
| RGJ-4.5 | 23.8 e | 19.6 c | 14.6 c | 11.2 c | 9.1 e | 8.4 e | 8.2 e | 8.3 e | 8.2 f | 8.1 f |
| CDGJM-9 | 22.6 h | 17.3 f | 11.1 fg | 7.5 g | 7.7 h | 7.6 f | 7.6 f | 7.6 f | 7.7 g | 7.6 g |
| RGJ-9 | 24.1 d | 19.6 c | 13.7 d | 10.5 d | 8.9 f | 8.7 d | 8.6 d | 8.6 d | 8.6 e | 8.4 e |
| CDGJM-18 | 23.4 g | 17.4 f | 11.2 fg | 8.4 f | 8.7 g | 8.7 d | 8.7 d | 8.8 d | 8.9 d | 8.6 d |
| RGJ-18 | 24.6 b | 19.6 c | 13.5 de | 10.4 d | 9.5 d | 9.5 c | 9.3 c | 9.3 c | 9.2 c | 9.3 c |
| CDGJM-36 | 24.3 c | 17.7 e | 11.4 f | 9.7 e | 9.9 c | 9.8 b | 9.8 b | 9.8 b | 9.7 b | 9.8 b |
| RGJ-36 | 25.9 a | 20.3 a | 13.3 e | 11.1 c | 11.1 a | 11.0 a | 10.9 a | 10.9 a | 10.8 a | 10.7 a |

Means within a column followed by different letters are significantly different ($p < 0.05$)
Significance level at $p < 0.05$, data analyzed by one-way ANOVA, Fisher's LSD

**Table S2.** Impact of *GL* on liking, perceived sensory attribute overall intensity and CATA ratings from benchtop evaluations of commercial Shiraz wine spiked with *GL* (panel n = 32) and medium-scale pre-fermentation *GL* wines (panel n = 11).

**Commercial Shiraz wine spiked with *GL***

| | | Overall intensity | | | | | | | CATA | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Wine** | **Liking** | **Aroma** | **Sweetness** | **Acidity** | **Hotness** | **Umami** | **Bitterness** | **Astringency** | **Earthy aroma*** | **Mushroom aroma*** | **Red berry aroma*** | **Oak aroma*** |
| 0 | 5.25 a | 4.16 b | 2.51 ab | 4.45 | 4.25 | 2.93 | 4.03 b | 4.06 | 22b | 9c | 53 | 47a |
| 2.25 | 5.17 a | 4.46 ab | 2.96 ab | 4.34 | 4.37 | 3.18 | 3.90 b | 3.93 | 13b | 22bc | 53 | 22ab |
| 4.5 | 4.72 ab | 4.43 ab | 3.00 a | 4.31 | 4.40 | 3.34 | 4.40 b | 4.06 | 25b | 38abc | 56 | 19b |
| 6.75 | 4.48 ab | 4.53 ab | 2.28 b | 4.18 | 4.12 | 3.59 | 4.56 b | 3.93 | 34ab | 44ab | 31 | 19b |
| 9.0 | 4.06 b | 5.00 a | 2.53 ab | 4.06 | 4.09 | 3.56 | 5.28 a | 4.18 | 56a | 56a | 28 | 28ab |

**Medium-scale pre-fermentation *GL* wines**

| | | Overall intensity | | | | | | | CATA | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Wine** | **Liking** | **Aroma** | **Sweetness** | **Acidity** | **Hotness** | **Umami** | **Bitterness** | **Astringency** | **Floral flavor*** | **Tropical flavor*** | **Lychee flavor*** |
| 0 | 4.27 | 5.54 | 6.27 a | 4.00 b | 2.18 b | 2.36 | 2.36 b | 2.50 | 55a | 73 | 73a |
| 4.5 | 4.90 | 5.40 | 3.00 b | 5.80 a | 3.80 a | 3.30 | 3.80 a | 3.30 | 0b | 36 | 9b |
| 9.0 | 4.44 | 5.67 | 3.22 b | 5.56 a | 3.78 a | 3.56 | 4.33 a | 3.22 | 18ab | 27 | 9b |

Values for liking and aroma and palate attribute overall intensities are mean values. Means within a column followed by different letters are significantly different ($p < 0.05$) analyzed by one-way ANOVA, with Fisher's LSD. *Only significantly different attributes from the CATA evaluations shown as selection frequency percentage based on Cochran's Q tests are presented. Sheskin critical difference pairwise comparison was used to test significant differences between wine treatments and indicated by lower case letters.

**Table S3.** Significant sensory attribute intensity means of *GL* wines (from 28 L ferments) generated by RATA panel.

| Sensory attributes | Control | POST 1 | POST 4 | PRE 1 | PRE 2 | PRE 4 |
|---|---|---|---|---|---|---|
| Ap-red | 4.2 a | 4.0 b | 3.9 b | 4.0 b | 4.1 b | 3.7 c |
| Ap-brown | 2.3 d | 2.8 c | 3.7 a | 2.5 d | 2.9 c | 3.4 b |
| A-red fruit | 3.7 a | 3.3 b | 2.9 c | 3.6 a | 3.5 ab | 3.0 c |
| A-dried fruit | 1.6 d | 1.8 cd | 2.2 a | 1.6 d | 1.9 bc | 2.1 ab |
| A-jammy | 2.0 ab | 2.1 a | 2.0 ab | 1.7 b | 2.0 a | 2.0 ab |
| A-confectionery | 2.9 ab | 2.7 bc | 2.1 e | 3.0 a | 2.5 cd | 2.3 de |
| A-cooked vegetable | 0.4 b | 0.4 b | 0.6 a | 0.3 b | 0.5 ab | 0.7 a |
| A-earthy | 0.7 c | 0.8 bc | 1.1 a | 0.7 c | 1.0 a | 1.0 ab |
| A-floral | 2.2 a | 2.2 a | 1.8 b | 2.2 a | 2.0 ab | 1.7 b |
| A-mushroom | 0.4 c | 0.5 bc | 0.8 a | 0.4 c | 0.6 ab | 0.7 ab |
| A-leather | 0.5 abc | 0.5 bc | 0.7 ab | 0.4 c | 0.6 ab | 0.7 a |
| A-savory | 0.7 b | 0.7 b | 1.2 a | 0.7 b | 1.0 a | 1.1 a |
| A-spice | 1.4 ab | 1.3 b | 1.5 a | 1.4 ab | 1.4 ab | 1.2 b |
| A-toasty | 0.8 cd | 0.8 d | 1.4 a | 0.8 d | 1.0 bc | 1.1 b |
| A-woody | 0.8 bc | 0.9 bc | 1.2 a | 0.7 c | 1.0 ab | 1.0 ab |
| A-tobacco | 0.5 c | 0.6 bc | 0.9 a | 0.6 bc | 0.6 bc | 0.7 b |
| T-bitter | 3.0 b | 3.2 b | 3.6 a | 3.1 b | 3.5 a | 3.7 a |
| T-sweet | 2.7 a | 2.6 a | 2.5 ab | 2.5 ab | 2.7 a | 2.4 b |
| T-sour | 2.7 b | 2.8 ab | 2.8 ab | 2.9 ab | 2.7 b | 3.0 a |
| F-dark fruit | 1.4 bc | 1.6 ab | 1.8 a | 1.4 c | 1.6 abc | 1.6 abc |
| F-red fruit | 3.5 ab | 3.3 b | 2.9 c | 3.6 a | 3.2 b | 2.9 c |
| F-dried fruit | 1.4 c | 1.6 bc | 2.0 a | 1.4 a | 1.7 ab | 1.8 ab |
| F-jammy | 1.7 a | 1.9 a | 1.9 a | 1.4 b | 1.9 a | 1.6 ab |
| F-confectionery | 2.4 a | 2.2 ab | 2.0 bc | 2.3 a | 2.2 abc | 1.9 c |
| F-cook vegetable | 0.4 ab | 0.4 ab | 0.5 a | 0.3 b | 0.5 ab | 0.5 ab |
| F-earthy | 0.7 cb | 0.7 bcd | 1.0 a | 0.7 d | 0.9 ab | 0.9 abc |
| F-floral | 1.9 a | 1.8 a | 1.5 b | 1.8 a | 1.8 a | 1.5 b |
| F-mushroom | 0.4 c | 0.4 bc | 0.7 a | 0.4 bc | 0.6 ab | 0.6 a |
| F-green capsicum | 0.5 ab | 0.4 b | 0.6 ab | 0.5 ab | 0.5 b | 0.6 a |
| F-herbaceous | 1.1 ab | 0.9 b | 1.1 ab | 1.0 ab | 1.0 ab | 1.2 a |
| F-leather | 0.4 bc | 0.5 abc | 0.7 a | 0.4 c | 0.6 ab | 0.7 a |
| F-pepper | 0.7 ab | 0.6 b | 0.8 ab | 0.6 b | 0.8 a | 0.7 ab |
| F-savory | 0.6 c | 0.8 bc | 1.0 ab | 0.7 c | 1.1 a | 1.0 ab |
| F-spice | 1.4 bc | 1.4 bc | 1.6 a | 1.3 c | 1.6 ab | 1.4 abc |
| F-toasty | 0.7 d | 0.9 bcd | 1.1 ab | 0.8 cd | 1.0 abc | 1.2 a |
| F-woody | 0.8 c | 0.9 c | 1.2 a | 0.9 bc | 0.9 bc | 1.1 ab |
| F-tobacco | 0.5 b | 0.6 b | 0.9 a | 0.5 b | 0.8 a | 0.8 a |
| M-body | 3.3 b | 3.4 ab | 3.5 a | 3.3 ab | 3.4 ab | 3.3 ab |
| M-alcohol/heat | 3.7 ab | 3.8 ab | 3.9 a | 3.7 b | 3.9 a | 3.9 a |
| M-astringency | 2.6 bc | 2.8 ab | 2.8 ab | 2.5 c | 2.7 ab | 2.8 a |
| M-smoothness | 3.5 a | 3.3 abc | 3.2 bc | 3.4 ab | 3.3 abc | 3.1 c |
| M-roughness | 2.4 bc | 2.6 ab | 2.6 ab | 2.3 c | 2.5 abc | 2.7 a |
| FL-fruit | 3.5 a | 3.6 a | 3.2 c | 3.4 ab | 3.5 ab | 3.3 bc |
| FL-non-fruit | 3.5 cd | 3.5 bcd | 3.7 ab | 3.4 d | 3.7 abc | 3.8 a |

Means within a row followed by different letters are significantly different. Significance level at $p < 0.05$, data analyzed by one-way ANOVA, Fisher's LSD. Prefix A- = aroma attribute, T- = taste; F- = flavor attribute, M- = mouthfeel, Ap- = appearance, FL- = aftertaste (fruit and non-fruit). Prefix PRE = *GL* added prior to fermentation (PRE 1, PRE 2 and PRE 4) and POST = *GL* added after fermentation (POST 1 and POST 4).

# Appendix

## List of attributes used in Rate-all-that apply sensory evaluation

| Aroma | Flavour | Appearance | Palate |
|---|---|---|---|
| A-dark fruit | F-dark fruit | Ap-red | M-body |
| A-red fruit | F-red fruit | Ap-brown | M-alcohol/heat |
| A-dried fruit | F-dried fruit | | M-astringency |
| A-jammy | F-jammy | | M-smoothness |
| A-confectionery | F-confectionery | | M-roughness |
| A-coconut | F-coconut | | T-bitter |
| A-cooked vegetables | F-cooked vegetables | | T-sweet |
| A-earthy | F-earthy | | T-sour |
| A-eucalypt | F-eucalypt | | |
| A-floral | F-floral | | |
| A-mushrooms | F-mushrooms | | |
| A-green capsicum | F-green capsicum | | |
| A-herbaceous | F-herbaceous | | |
| A-leather | F-leather | | |
| A-pepper | F-pepper | | |
| A-savoury | F-savoury | | |
| A-spice | F-spice | | |
| A-toasty | F-toasty | | |
| A-sweet-oak | F-sweet-oak | | |
| A-woody | F-woody | | |
| A-tobacco | F-tobacco | | |

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# CERTIFICATE of PUBLICATION

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Volatile Composition and Sensory Profiles of a Shiraz Wine Product Made with Pre- and Post-Fermentation Additions of *Ganoderma lucidum* Extract

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# Chapter 4

# Sensory and chemical drivers of wine consumers' preference for a new Shiraz wine product containing *Ganoderma lucidum* extract as a novel ingredient

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Submitted to Foods in 2019.

# Statement of Authorship

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|---|---|
| Title of Paper | Sensory and chemical drivers of wine consumers' preference for a new Shiraz wine product containing *Ganoderma lucidum* extract as a novel ingredient |
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## Principal Author

| | |
|---|---|
| Name of Principal Author (Candidate) | Anh N. H. Nguyen |
| Contribution to the Paper | Designed and translated the questionnaire using Surveymonkey®, performed RATA sensory tasting sessions using Redjade (served six wines and recruited 124 participants) at the central market (Adeladie, South Australia).<br>Conducted wine chemical and sensory analyses. Assisted wine volatile analysis (with HS-SPME-gas chromatography mass spectrometry), and interpreted data. Constructed, edited and revised the manuscript. |
| Overall percentage (%) | 70 |
| Certification: | This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper. |
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## Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);

ii. permission is granted for the candidate in include the publication in the thesis; and

iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

| | |
|---|---|
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| Contribution to the Paper | Analysed and interpreted collected data (chemical and sensory data using SENPAQ).<br>Performed the hedonic clustering based on individual liking scores for each wine and wine knowledge segmentation.<br>Analysed sensory and volatile flavour that significantly differentiated the wines and analysed those significant variables in Partial Least Squares Regression modelling using XLSTAT.<br>Mainly contributed to the first draft's content. Critically assisted with editing and revising the manuscript. |
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*Article*

# Sensory and chemical drivers of wine consumers' preference for a new Shiraz wine product containing *Ganoderma lucidum* extract as a novel ingredient

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**Abstract:** This study explored wine consumers' preference towards a novel Australian Shiraz wine product containing *Ganoderma lucidum* (*GL*). Wine consumers (n = 124) completed a questionnaire and tasted six *GL* wine products (differing in amount and timing of *GL* extract additions). Based on liking scores for each product tasted, three hedonic clusters (C1, C2, C3) were identified. Sensory attributes of the *GL* wine products were profiled with rate-all-that-apply (n = 65) and the 31 sensory attributes that significantly differentiated the wines underwent Principal Component Analysis with the hedonic clusters overlaid. A clear separation between hedonic clusters indicated C1 preferred red appearance, pepper, red fruit, cooked vegetable, earthy, mushroom, leather and woody aromas, and pepper, green capsicum, spice, cooked vegetable, mushroom and floral flavors, and more astringent mouthfeel. In contrast, C2 liked brown appearance and tobacco aromas and C3, wines with floral and confectionery aromas, red-fruit notes and smooth mouthfeel. Sensory attributes and volatile flavor compounds that significantly differentiated the wines, were subjected to partial least squares regression, which indicated the important positive drivers of liking among the hedonic clusters. These findings provide the wine industry with deeper insights into consumers' liking towards new *GL* wine products targeted at the Australasian market.

**Keywords:** hedonic clusters, rate-all-that-apply (RATA), medicinal mushroom, wine volatile chemistry.

## 1. Introduction

*Ganoderma lucidum* (*GL*), a highly revered curative mushroom, has been commonly used for more than 2000 years, due to its promotion of longevity and prevention of diseases [1-5], which has drawn the attention of researchers. Furthermore, both researchers and consumers from different countries, including some Asian countries, North America and Europe, are interested in the application of *GL* in the production of health-supporting products [6]. For example, in China, the presence of *GL* extract during the fermentation process of soy milk improved the health benefits as well as the consumer acceptability of the product [7]. Other products, such as Chinese *GL lycium chinensis miller* tomato wine [8], and yogurt [9,10] with *GL* extract added during production, have also been described. Alcoholic beverages have even been investigated; Serbian Pilsner beer and brandy had *GL* added aseptically to create a better-perceived body in the beer [11] and a brandy [12] with functional properties. Recently, Ghobadi, *et al.* [13] demonstrated that *GL* powder is an effective preservative in the production of sausage for the meat industry. However, to the best of our knowledge, there is currently no study on

the development of a novel *GL* grape wine product for the Australian and its potential global wine markets.

There is no doubt that every consumer has a unique pattern of taste preference, resulting in consumers displaying a diverse range of behaviors [14] that can be difficult to interpret but could be due to differences in socio-demographics such as individual knowledge, cultural background, age, gender, etc. Market segmentation is a technique identifying consumers within a specific market who have similar wants, needs and behaviors [15]. Meilgaard, *et al.* [16] suggested that consumers with similar attitudes might be identified by cluster analysis. Many segmentation bases have been applied in the literature, with examples for wine consumers that include liking scores [17-19], knowledge or product-related experience [20,21], demographics [22] and psychographic segmentation bases such as personality, values or interest. Besides the application of geographic, demographic and socio-economic, behavioral, and psychographic segmentation bases in the wine market, other segmentation bases in various wine market models are emerging, including those based on biology, sustainability and social media [23]. Notably, consumer acceptance and behavior studies appear to favor hedonic clustering whereas marketing-focused research is more aligned with the other criteria such as geographic, demographic, and so on. However, the combination of these two distinct bases can provide powerful additional insights into consumer responses [14,24].

There is only one consumer study relating consumers' attitudes toward a new Australian wine product containing *GL* reported by Nguyen, *et al.* [25]. This research revealed that although consumers from all three nations under examination were willing to try *GL* wine products, Vietnamese consumers were more interested in these wine products compared to Australian and Chinese consumers. Indeed, despite the promise of wine supplemented with traditionally revered foods such as *GL*, no studies have focused on consumers' acceptance of *GL* wine products, including from the perspective of individual liking, and demographic differences in conjunction with sensory and chemical profiling.

To profile products' sensory characteristics, numerous studies have proposed several sensory methods that are suitable for naïve consumers such as napping, free choice profiling, flash profiling, check-all-that-apply (CATA), and rate-all-that-apply (RATA) [26-28]. According to Jaeger and Ares [29] and Danner, Crump, Croker, Gambetta, Johnson and Bastian [28], compared to other rapid consumer methods, the RATA method is more easily understood by consumers and less likely to bias their hedonic score. RATA is an economical and flexible sensory method, suitable for profiling a variety of products including wine [28]. This study aimed to investigate the sensory attributes and chemical composition driving consumer clusters' liking of *GL* wine products using a consumer-centric, new product development approach. A combination of a rapid sensory profiling method using naïve consumers (RATA), basic analytical chemistry and volatile chemistry analysis using Headspace-solid-phase micro-extraction (HS-SPME), Gas chromatography-mass spectrometry (GC-MS) followed by consumer acceptance testing and hedonic segmentation was employed.

Responding to these gaps in the knowledge, this study aimed to investigate:

- Consumers' acceptance of novel *GL* wine products.
- The most preferred level of *GL* extract added prior to or post-fermentation in the production of these novel wines.
- Consumer profiles of *GL* wine hedonic clusters.
- The sensory attributes in *GL* wine products important for driving consumer hedonic cluster responses.
- The identification of the sensory and chemical parameters most important to consumer *GL* wine products liking using prediction models.

This knowledge might assist to increase the competitiveness of the Australian wine industry locally and in its many wine export countries.

## 2. Materials and Methods

### *2.1 Samples*

Six Australian Shiraz wine products made with and without *GL* extract added at different amounts and stages of the fermentation process described in Nguyen, *et al.* [30] were used for a blind consumer preference test in the current study. These six products were selected from the original 18 wines (six wines produced in triplicate ferments) based on their RATA sensory profiles, because they were a good representative of the three replicates within a treatment and clearly discriminated from one another. Based on the Australian and New Zealand Food Standards (Standard 2.7.4), such wines containing *GL* would be considered a "wine product" but will be referred to as wine throughout the remainder of the text for simplicity.

The samples included wines fermented with three different levels of *GL* extracts added prior to inoculation of yeast for primary fermentation, namely 1 g/L (PRE 1), 2 g/L (PRE 2) and 4 g/L (PRE 4); wines enriched with two levels of *GL* after fermentation, being 1 g/L (POST 1) and 4 g/L (POST 4), and a wine without addition of *GL* (Control). All wine samples were stored at 15 °C and acclimatized to room temperature (22-23 °C) a day before serving.

### *2.2 Chemical and sensory measurements*

#### 2.2.1 Basic wine composition

Basic chemical parameters were measured in a previous study [30]. Briefly, volatile acidity (VA), and free and total $SO_2$ content were determined using the methods described in Iland, *et al.* [31]. Measurements of pH and titratable acidity (TA), wine color (CIELAB) and ethanol content (% v/v) were undertaken with a T50 Titrator (Mettler-Toledo, Port Melbourne, Victoria, Australia), Cintra 4040 (GBC Scientific Equipment, Victoria, Australia) and Alcolyzer ME/DMA 4500 M (Anton Paar, Graz, Austria), respectively. Residual sugars were determined enzymatically with a Chemwell 2910 auto-analyzer following the procedure for K-FRUGL kits (Megazyme, Wicklow, Ireland). Only VA was measured in duplicate, other measurements of wine parameters were conducted in triplicate.

#### 2.2.2 HS-SPME GC-MS analysis of wines

HS-SPME coupled with GC-MS was used to identify and quantify key volatiles in *GL* wines. Twenty-nine volatile compounds reported in the previous study of [30] were used for PLS analysis in conjunction with sensory data from the current study.

#### 2.2.3 Rate-all-that-apply (RATA) sensory panel

The Shiraz wines were assessed by 65 participants who were aged 28 to 35 years (50.7% female), and had consumed wine in the last 12 months. The sensory panels used RATA with a seven-point intensity scale, anchored from 1 = "extremely low" to 7 = "extremely high". RATA evaluations were conducted as described in Danner, Crump, Croker, Gambetta, Johnson and Bastian [28].

### *2.3 Consumer sample*

Wine consumers (n = 124, 52% female) were recruited at a food market in Adelaide, South Australia, where the service of alcohol to consumers was permitted through a limited alcohol license obtained by the researchers. Inclusion criteria required respondents to be of legal drinking age (i.e., above 18 years old) and to have consumed grape wine within the past 12 months. This study was approved by the Human Research Ethics Committee of The University of Adelaide (Approval No. H-2016-194).

### *2.4 Questionnaire*

Survey Monkey™ (Palo Alto, CA, USA; http://www.surveymonkey.com) was used for the consumer questionnaire, which consisted of five sections that covered demographic information, alcohol consumption behavior, consumer behavior towards *GL* wines and a blind tasting of the *GL* wines. Section one, demographic questions, related to age, gender, education, household income, the preferred price for a bottle of wine, wine-drinking frequency, and the preferred place to purchase wine. The second section asked about wine purchase drivers with questions covering 14 factors used in choosing wines, where 1 = extremely unimportant, 5 = neither important nor unimportant and 9 =

extremely important. Section three included questions that asked respondents to rate their liking score for the six *GL* wine samples in the blind tasting. In the last section, participants were asked to rate their level of agreement on a series of statements with respect to their attitudes towards novel *GL* wines. As with the previous study [25], the questionnaire was generated in English and underwent a pilot test by a small group of staff (n = 4) from the School of Agriculture, Food and Wine at the University of Adelaide, to clarify any ambiguity before being used for the consumer trial.

The consumer tasting sessions were designed using RedJade® software (Redwood City, USA) to produce a packing slip with an identifier number for each person and to determine the presentation order of the six wines. Wines (30 mL) were served at room temperature on an individual white tray for each person in randomized order, in transparent 215 mL International Organization for Standardization wine glasses labelled with three-digit codes and covered with glass lids. Participants were required to read the participant information sheet and consent to participate in the study before tasting the wines. Respondents were instructed to score their liking for each wine on a nine-point Likert category scale anchored by 1 = extremely dislike, 5 = neither like nor dislike, and 9 = extremely like. To cleanse the palate, participants were offered unsalted crackers and water plus were required to have a one-minute break between samples. Participants could taste the same sample again if necessary but could not go back and re-taste the previous sample.

### *2.5 Data analyses*

The data were collected and analyzed by various tools: XLSTAT (Ver. 2017, Addinsoft, New York, USA), SPSS Statistic 24 (2013, IBM Corp, Armork, NY, USA) and SENPAQ (version 5.01, 2010, Qi statistics, UK,). Basic chemical data were analyzed by one-way Analysis of Variance (ANOVA) with Tukey's HSD, post-hoc test using SPSS. SENPAQ was used to analyze sensory data, by a mixed model ANOVA with assessors as random factors and Fisher's LSD post-hoc test for multiple comparisons. Thirty-one significant sensory attribute means and hedonic clusters as supplementary data were subjected to Principal Component Analyses (PCA) using XLSTAT, and a biplot of cluster data and sensory attributes was generated using XLSTAT. For hedonic clustering, liking scores were subjected to a k-means cluster analysis in XLSTAT. Chi-square tests were used to test for differences in demographics between the three identified hedonic clusters using XLSTAT. Differences in mean overall liking and liking between the three clusters were determined with a one-way ANOVA using LSD post-hoc tests. Volatiles were also analyzed by one-way ANOVA with LSD post-hoc test using XLSTAT. Finally, significantly different volatile chemical components and sensory attributes were subjected to partial least squares (PLS) regression for hedonic clusters using XLSTAT. The variables considered important for the prediction model were chosen based on Variable Importance in the Projection VIP values > 0.8; sensory and chemical data were used as the predictor X variables and the cluster mean liking scores as the Y variables in PLS analysis [24]. All the statistical tests were conducted using a significance level of 0.05.

## **3. Results**

In this study, *GL* wines were made for the first time. However, before potential release to the market, there was a need to examine consumers' preferences in more detail and how they related to the wines' sensory and chemical profiles. This may provide Australian winemakers with initial insights into the formulation of a novel wine product that might appeal to various markets.

### *3.1. Consumer preference*

#### 3.1.1. Overall liking

Liking scores for *GL* wines are presented in Table 1 showing the liking ratings ranged from 4.1 to 5.2. These values are in accordance with previous studies with Australian consumers reporting mean liking ratings between 5 and 6 for high-quality commercial wine samples during a blind tasting [32,33]. This indicates that Australian consumers might be cautious when scoring wines tasted blind,

and higher ratings might only observed when additional information about the wines gets presented together with the samples [33].

The mean hedonic data revealed statistical differences in the liking of both Control and the POST 1 g/L *GL* wine compared with that of the wines with higher *GL* additions (2 and 4 g/L) (Table 1). This was in line with a previous study conducted by Kim, *et al.* [34] who, using 50 trained panelists, demonstrated that Korean rice wine, Yakju, with *GL* added at 1 g/L was the most acceptable, but wines with higher levels of *GL* extracts resulted in an unfavorable taste due to the enhanced bitterness. Indeed, the Shiraz wines with higher amounts of *GL* extract (4 g/L) added PRE or POST fermentation were perceived as more bitter (Figure 1). In contrast, Pecic, *et al.* [35] reported that commercial grape brandy with 25 g/L *GL* addition had the best acceptability following sensory evaluation by five qualified experts. It is possible that unlike brandy, bitter wine compounds such as flavan-3-ols [36] present in the wines of the current study may have interacted with the relatively low levels of *GL* and had an additive effect on perceived bitter taste. Further investigation of the red wine polyphenolic and *GL* bitter compound content of *GL* red wines, and estimation of their rejection thresholds in red wine matrices, may provide useful information for future product development projects in terms of the determination of *GL* levels that best suit consumers' acceptability towards various kinds of *GL* foods and beverages. The wines in the current study were also made from red juice and not fermented with skins and seeds as is usually the case for dry red table wines. Fermentation of *GL* wines on skins would likely produce more full-flavored wines with higher color and tannin [37], which would also modulate the impact of *GL* additions and potentially enhance consumer acceptability [38].

**Table 1.** Consumers' overall liking means for the whole cohort and by consumer clusters of six *GL* wines.

| Wine samples | Overall liking | Cluster 1 (n = 41) | Cluster 2 (n = 37) | Cluster 3 (n = 46) |
|---|---|---|---|---|
| Control | 5.2 a (0.181) | 5.4 a | 4.3 cd | 5.9 a |
| POST 1 | 5.2 a (0.181) | 3.7 c | 6.0 a | 6.0 a |
| PRE 1 | 5.1 ab (0.181) | 3.9 bc | 5.4 ab | 5.9 a |
| PRE 2 | 4.6 bc (0.181) | 5.3 a | 3.6 d | 4.9 b |
| POST 4 | 4.5 cd (0.181) | 4.6 ab | 5.6 a | 3.7 c |
| PRE 4 | 4.1 d (0.181) | 4.8 ab | 4.7 bc | 3.0 d |

Data were collected with a nine-point hedonic Likert category scale anchored by 1 = extremely dislike, 5 = neither like nor dislike, and 9 = extremely like and analyzed by one-way ANOVA, with Fisher's LSD post-hoc tests, with a significance level of $p < 0.05$. Different letters within a column indicate significant differences between samples' liking scores. Standard errors in parentheses.

3.1.2. Cluster analysis of consumers' hedonic scores

In order to identify consumer clusters with different preferences for each of the *GL* wines, consumers were asked to report their liking for each *GL* wine on a nine-point hedonic category scale in the blind tasting. The k-means cluster analysis was performed and a three-cluster solution was revealed, with discriminant analysis yielding a 98% accurate fit for the data set. The resultant clusters were: cluster 1 (C1, n = 41, 33.06%), cluster 2 (C2, n = 37, 29.84%) and cluster 3 (C3, n = 46, 37.10%). C1 preferred wines made without and with 2 g/L *GL* added before fermentation (Control and PRE 2, mean liking scores of 5.4 and 5.3, respectively) (Table 1). C2 consisted of respondents who liked all the wines with 1 g/L *GL* addition (mean liking scores of 5.4 and 5.9 for PRE 1 and POST 1, respectively) and wine with *GL* 4 g/L added after fermentation (mean liking score of 5.6). Finally, C3 preferred Control, and wines supplemented with 1 g/L *GL* (Control, PRE 1 and POST 1) but disliked wines with higher *GL* additions (2 and 4 g/L). Knowing the influence that polymorphisms of genes responsible for bitter taste have on consumer food choice and preference [39], it would be of interest to examine the consumer cluster's bitter plus other orosensory phenotypes and genotypes to see if this impacted cluster *GL* wine preferences. Furthermore, in future, analyses of the wine polyphenolic profile and the *GL* extract bitter compound composition could provide more cluster liking insights. Perceived bitterness from *GL* addition may be moderated by retaining small amounts of residual

228 sugars at the end of primary fermentation or adding grape juice concentrate post-ferment [37], which
229 would render the wines more palatable to more consumers.
230 3.1.3. Demographics of hedonic clusters
231 Table 2 illustrated that there were no significant demographic differences between hedonic
232 clusters. When asked various statements with respect to *GL* wines after the blind tasting, no
233 significant differences in consumer behavior were found except for the statement "I am keen on
234 drinking *GL* wine if the price is reasonable" ($p = 0.039$), where C3 responses were significantly higher
235 than C1 (mean scores 6.0 and 4.8, respectively) (Table 3).

236 **Table 2.** Demographics of three hedonic clusters.

| | Total (n = 124) | Cluster 1 (n = 41) | Cluster 2 (n = 37) | Cluster 3 (n = 46) |
|---|---|---|---|---|
| **Gender** | | | | |
| Male | 47.6 | 51.2 | 45.9 | 45.7 |
| Female | 52.4 | 48.8 | 54.1 | 54.3 |
| **Age** | | | | |
| 18-34 | 41.9 | 31.7 | 46.0 | 47.8 |
| 35-54 | 33.1 | 34.2 | 32.4 | 32.6 |
| +55 | 25.0 | 34.1 | 21.6 | 19.6 |
| **Education** | | | | |
| Non-tertiary | 42.7 | 39.0 | 40.5 | 47.8 |
| Bachelor's degree | 29.0 | 31.7 | 27.1 | 28.3 |
| Post-graduate degree | 28.3 | 29.3 | 32.4 | 23.9 |
| **Household income (AU$)** | | | | |
| <$50,000 | 52.4 | 41.5 | 51.4 | 63.0 |
| $50,001-$100,000 | 29.8 | 36.6 | 35.1 | 19.6 |
| $100,001-$200,000 | 12.9 | 17.0 | 5.4 | 15.2 |
| >$200,000 | 4.9 | 4.9 | 8.1 | 2.2 |
| **Price per 750 mL bottle of wine (AU$)** | | | | |
| less than $15 | 22.6 | 17.1 | 24.3 | 26.1 |
| $15-$29 | 53.2 | 56.1 | 59.5 | 45.7 |
| $30-$49 | 19.4 | 22.0 | 13.5 | 21.7 |
| $50-$100 | 1.6 | 2.4 | 2.7 | 0.0 |
| More than $100 | 1.6 | 2.4 | 0.0 | 2.2 |
| Never purchase | 1.6 | 0.0 | 0.0 | 4.3 |
| **Wine consumption frequency** | | | | |
| Few times per week | 50.0 | 53.7 | 51.4 | 45.6 |
| Once per week | 16.9 | 17.0 | 13.5 | 19.6 |
| Once per two weeks | 13.7 | 19.5 | 10.8 | 10.9 |
| Once per month | 19.4 | 9.8 | 24.3 | 23.9 |
| **Place of wine purchase** | | | | |
| Online wine/liquor store | 8.9 | 17.0 | 2.7 | 6.5 |
| Wineries/cell door | 12.9 | 9.8 | 16.2 | 13.1 |
| Retail chain liquor store | 66.1 | 61.0 | 70.3 | 67.4 |
| Independent wine store | 3.2 | 4.9 | 2.7 | 2.2 |
| Restaurant | 4.0 | 2.4 | 5.4 | 4.3 |
| Others (clubs, bars, hotels) | 4.9 | 4.9 | 2.7 | 6.5 |

Data presented are percentages. Chi-square values for wine hedonic clusters of the demographic data were: gender, $X_2 = 0.326$, df = 2, $p = 0.850$; age, $X_2 = 3.652$, df = 4, $p = 0.455$; education, $X_2 = 1.165$, df = 4, $p = 0.884$, income, $X_2 = 8.386$, df = 6, $p = 0.211$, preferred price for a 750 mL bottle of wine, $X_2 = 8.050$, df = 10, $p = 0.624$; wine consumption frequency, $X_2 = 5.163$, df = 6, $p = 0.523$; place to purchase wine, $X_2 = 7.481$, df = 10, $p = 0.679$.

237 **Table 3.** Australian consumers' attitude towards *GL* wines statements and their market expectations.

| | Wine hedonic clusters | | | | | | p-value |
|---|---|---|---|---|---|---|---|
| | Cluster 1 (n = 41) | | Cluster 2 (n = 37) | | Cluster 3 (n = 46) | | |
| | Mean | Std. error | Mean | Std. error | Mean | Std. error | |
| I do not know about the *GL* wine but I think it is worth trying. | 5.9 | 0.364 | 5.2 | 0.383 | 6.2 | 0.343 | 0.148 |
| I would like to go to places where *GL* wines are served. | 4.7 | 0.315 | 4.6 | 0.332 | 5.0 | 0.298 | 0.610 |
| I would drink almost any *GL* wine. | 4.0 | 0.328 | 4.2 | 0.345 | 4.5 | 0.309 | 0.538 |
| At a social gathering, I will try *GL* wine. | 6.8 | 0.293 | 6.3 | 0.308 | 6.4 | 0.276 | 0.568 |
| I am keen on drinking *GL* wine if the price is reasonable. | 4.8 b | 0.343 | 5.7 ab | 0.361 | 6.0 a | 0.324 | **0.039** |
| Not sound "romantic". | 5.6 | 0..348 | 4.8 | 0.367 | 4.8 | 0.329 | 0.147 |
| Are not as socially acceptable or impressive. | 5.0 | 0.343 | 4.3 | 0.361 | 4.0 | 0.324 | 0.074 |
| Should have this information specified on the label. | 7.1 | 0.320 | 6.6 | 0.337 | 6.3 | 0.302 | 0.163 |
| Does not matter to me as long as *GL* wine taste good. | 5.6 | 0.378 | 5.2 | 0.398 | 5.4 | 0.357 | 0.694 |
| Are the way of the future regarding health benefits. | 5.1 | 0.292 | 5.3 | 0.307 | 5.8 | 0.276 | 0.188 |
| Have no influence on my purchase decision. | 4.7 | 0.338 | 4.7 | 0.356 | 5.1 | 0.319 | 0.636 |
| Are cheap or of lower quality. | 4.8 | 0.274 | 4.5 | 0.260 | 5.0 | 0.246 | 0.427 |

Data presented are mean agreement scores; where 1 = highly disagree, 5 = neither agree nor disagree and 9 = highly agree. Different letters within a row indicate significant differences between wine hedonic clusters, data analyzed by one-way ANOVA, Fisher's LSD, significant level at $p < 0.05$.

238 *3.2. The relationship between hedonic clusters and sensory characteristics of GL wines*

239 Moussaoui and Varela [26] concluded that RATA - an economical and rapid sensory profiling
240 technique - was a suitable profiling method to use employing naïve consumers. This consumer RATA
241 panel identified 31 of 54 attributes (57%) ($p < 0.05$) as significantly differentiating the *GL* wines
242 (Supplementary Table S2). PCA was performed on the mean intensity ratings of the 31 significant
243 attributes, with the hedonic clusters overlaid as supplementary data (Figure 1). The first two principal
244 components (PCs) explained 83.67% of the variation in the data (PC1 (61.51%) and PC2 (22.16%)) and

revealed the relationships between these sensory attributes and each of the three hedonic clusters. PC1 separated wines (PRE 4 and POST 4) that had brown appearance, dried fruit and savory notes, earthy, leather, toasty and woody aromas, mushroom flavor, bitter taste, and astringent mouthfeel from those with confectionery and floral aromas and red fruit flavor (Control, PRE 1 and POST 1). Wines characterized by brown appearance, tobacco aroma, and rough mouthfeel were differentiated along PC2 from those that were red in appearance, had red fruit aroma and floral flavor, and a smooth mouthfeel. A clear separation was observed between hedonic clusters along both PC1 and PC2, with different sensory attributes driving hedonic scores for each cluster. C1 preferred *GL* wines with more red appearance, pepper, red fruit, cooked vegetable, earthy, mushroom, plus leather and woody aromas. This cluster also preferred spice, pepper, green capsicum, cooked vegetable, mushroom and floral flavors and a more astringent mouthfeel including the wines without (Control) and with 2 g/L *GL* added pre-fermentation (PRE 2). C2 preferred attributes such as brown appearance, tobacco aromas and the rougher mouthfeel in wines made with 4 g/L *GL* added post-fermentation (POST 4). C3 liked wines with 1 g/L *GL* added and without *GL* (Control, PRE 1, POST 1) and appeared especially accepting of floral and confectionery aromas, red-fruit notes along with a smooth mouthfeel. Considering the literature that has examined the sensory drivers and chemical composition of red wine consumer liking to date, there seem to be patterns emerging: i.e., those segments that like simple, red fruit, confected and smooth wines such as C3 in the current study; those who prefer wines with oak or oak-like notes, for example, the tobacco notes liked by C2 in the current study; and possibly another group who prefers more complex wines, with some texture plus spice, possibly green notes, and savory nuances, namely C1 as evident in the present work [24,40,41]. Using different *GL* concentrations in the winemaking process and also involving different grape varietals, oak fermentation or maturation, and varying cap management and fining treatments could develop a wider range of wines with new profiles characterized by the sensory attributes identified above, that potentially drive individual hedonic responses by consumers.

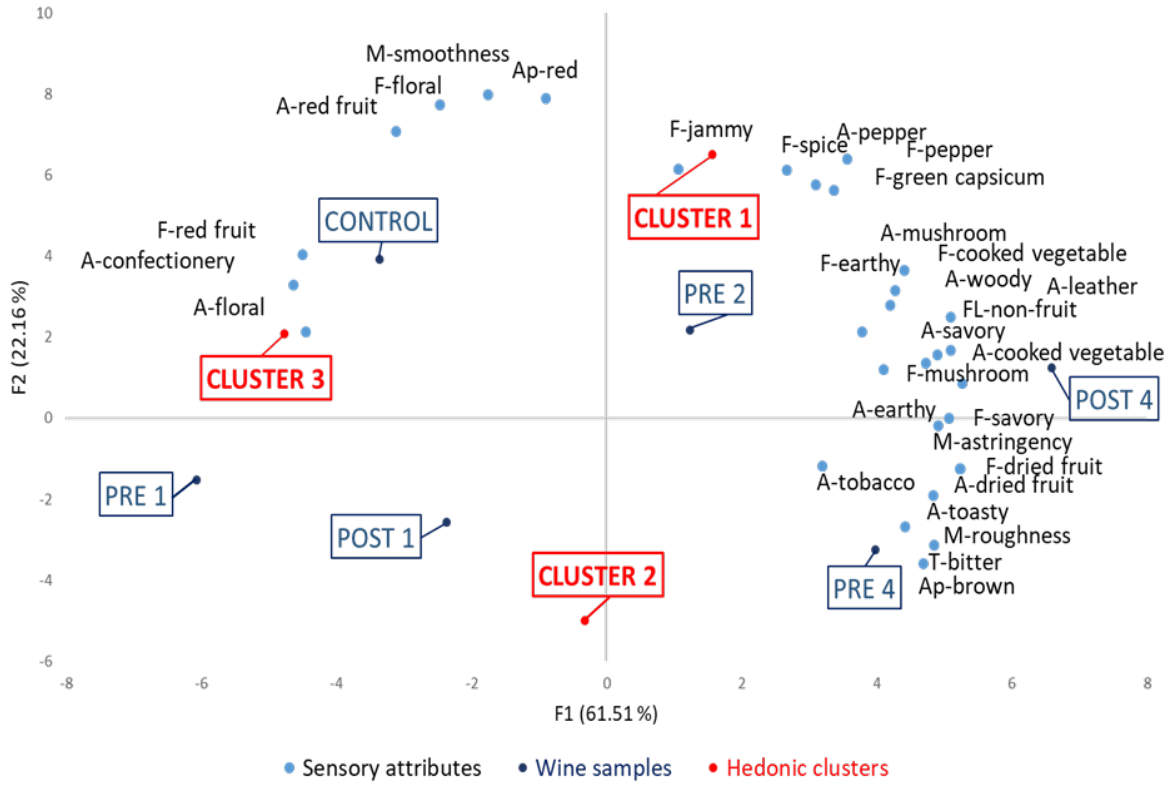


**Figure 1.** PCA of 31 sensory attributes perceived significantly different between six *GL* wines and three distinct hedonic consumer clusters as supplementary data, including Cluster 1 (n = 41); Cluster 2 (n = 37) and Cluster 3 (n = 46). Prefixes: A- = Aroma attribute; T- = taste; F- = flavor attribute; M- = mouth-feel, Ap- = appearance, FL- = aftertaste (fruit and non-fruit) intensity of different wine treatments, PRE = *GL* extracts added prior to fermentation (PRE 1, PRE 2 and PRE 4), and POST = *GL* extracts added after fermentation process (POST 1 and POST 4)

### *3.3. Sensory and chemical drivers of liking*

It is widely accepted that for commercial success, it is advantageous for wine producers to understand the sensory attributes that influence consumer preference for their wines. This is particularly pertinent in the context of the current study, as the prototype Australian Shiraz *GL* wines produced represented a novel concept. As such, it was not clear what the wines made with *GL* extracts would smell, taste and feel like, and the potential drivers of consumer liking or disliking of these novel wines were also unknown. Basic chemical composition parameters (Supplementary Table S1) were analyzed and found to significantly differentiate treatments, but as the differences between treatments were below perceived difference thresholds, they were not included in the final PLS analysis. Thus, 31 sensory attributes and eight volatile compounds (2-phenylethanol, 1-octanol, ethyl acetate, limonene, and hexanoic, octanoic, decanoic and 3-methylbutanoic acids) that significantly differentiated the wine treatments were subjected to PLS regression. The resultant regression standardized coefficients for the three clusters (C1, C2 and C3) are shown in Figure 2.

Drivers are considered the most important when the regression coefficients with absolute values are higher than 0.2 [24]. Figure 2 showed that most of the potential drivers including sensory attributes and volatile compounds did not predominantly influence the overall liking across the three hedonic clusters (regression coefficients < 0.1). Positive liking drivers of C1 were octanoic acid, green capsicum and spice flavors, plus non-fruit flavor aftertaste which, along with hexanoic acid, negatively drove liking of C2. Jammy flavor and tobacco aroma strongly drove C2's preference, while tobacco was important for C1's negative liking. The majority of drivers of C3 liking were related to negative coefficients, including 1-octanol, 3-methylbutanoic acid, hexanoic acid, brown appearance, dried fruit, cooked vegetable and savory notes, earthy, leather, toasty, woody aromas, bitter taste, mushroom flavor, astringent and rough mouthfeel and non-fruit flavor aftertaste (Figure 2) but none of them were considered highly important as the regression coefficients were less than -0.1. Although some sensory attributes had positive coefficients, including red fruit and floral notes and confectionery aroma, which aligned with a previous study reported by Wang, *et al.* [42], none of them strongly drove C3's liking in the present case because their regression coefficients were less than 0.1.

Three consumer clusters were identified, each with different positive and negative drivers of preference. When predicting the preference of the total consumer group, none of the variables strongly drove consumers' disliking (data not shown). This analysis reveals the potential for hedonic clustering techniques, married with other sensory and chemical data, to provide powerful consumer information that can be used in either developing new wine products, or better targeting consumer segments.

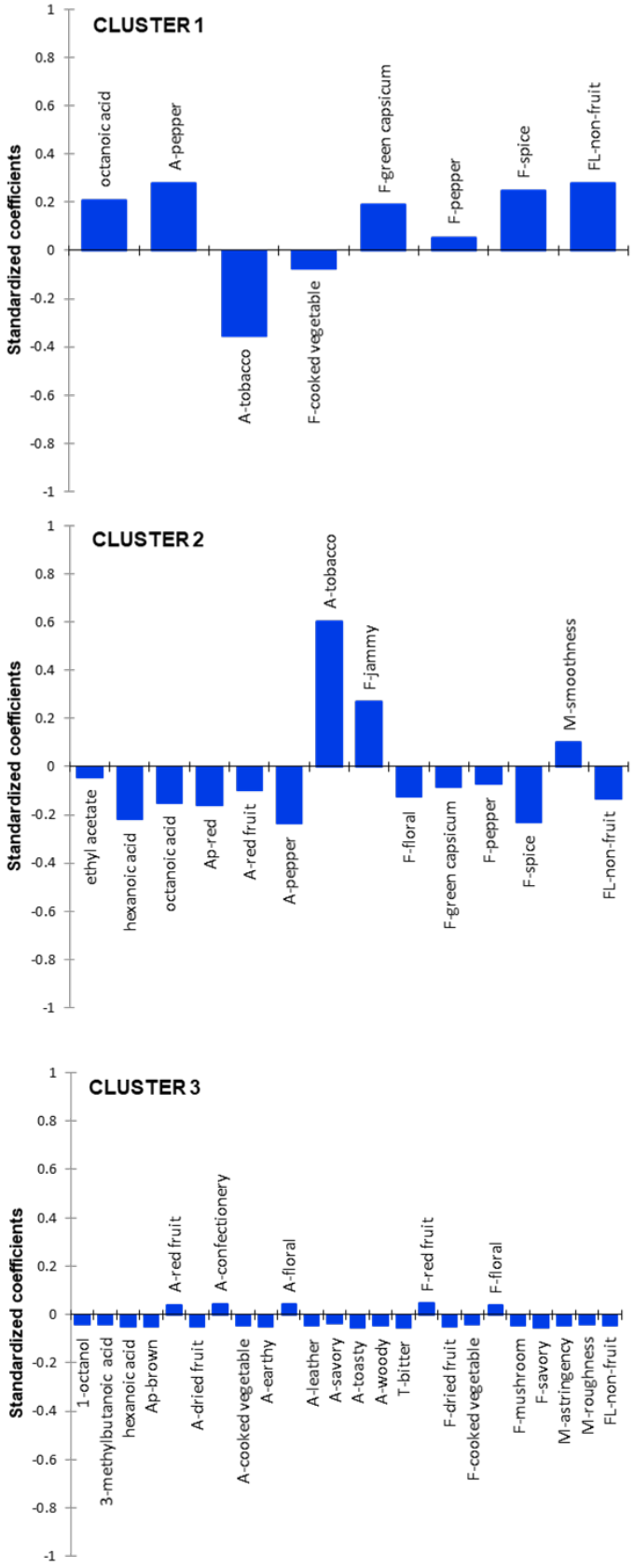


**Figure 2.** Standardized coefficients of the PLS regressions by hedonic cluster using liking scores as Y variables and sensory attributes and volatiles as X variables. Prefixes: A- = Aroma attribute; T- = taste; F- = flavor attribute; M- = mouth-feel, Ap- = appearance, FL- = aftertaste (fruit and non-fruit) intensity of different wine treatments. Drivers are considered the most important when the regression coefficients with absolute values are higher than 0.2.

## 4. Study limitations

The present study findings are subject to some limitations. Even though consumers were randomly invited to taste the wines at the market place, those that participated in the study were likely more interested in and involved with wine than regular consumers, which could lead to a higher level of wine knowledge as well as hedonic ratings. To commercialize new *GL* wine products, more standard red wine (and possibly white wine) production approaches would also need to be examined (i.e., making *GL* wines with skin contact in the fermentation, different grape varieties, oak contact, aging, and so on) to achieve styles of new *GL* wines for a target market. As only a limited number of volatile and sensory attributes were predicting cluster liking, it is possible other wine product components such as the polyphenols or *GL* extract compounds not measured in the current study could assist further explanation of preference.

## 5. Conclusions

Key barriers to the rapid success of Australian wines in Australasian markets are the lack of understanding of wine consumers' taste preferences and possibly the lack of involvement of consumers in the co-creation process of the wine. We aimed to investigate the development of a wine prototype for a specific target market, which evolved from the solicited consumer attitudes for *GL*-based wine presented in our previous work. Extending on that, RATA was used to assess consumer perceptions of Shiraz-based *GL* wine products, and sensory results in conjunction with wine composition were linked to the preference of consumer segments.

Although there were differences in hedonic scores for each *GL* wine corresponding to its sensory characteristics, most of the responses showed that consumers were very likely to be interested in the new Australian *GL* wines. The results of the study suggested that *GL* wines containing a low amount (i.e. 1 g/L) of *GL* extract, added either pre- or post-fermentation, were preferred and may form the basis for new wine products aimed at specific market segments. Better understanding of the chemical and sensory drivers of consumers' acceptance and preference for new *GL* wines, and incorporating consumers in the development process (i.e. co-creation), provided a novel approach that could help winemakers obtain deeper insights into the needs of a target market.

**Supplementary Materials:** The following are available online at www.mdpi.com/xxx/s1, Table S1: Basic chemical composition of the *GL* wines, Table S2: Mean intensity ratings of sensory attributes significantly differentiated six wines from RATA sensory test.

**Author Contributions:** Conceptualization, A.N.H.N., D.W.J. and S.E.P.B.; Formal analysis, A.N.H.N., D.L.C., T.E.J. and S.E.P.B.; Funding acquisition, S.E.P.B.; Investigation, A.N.H.N.; Methodology, D.L.C., T.E.J., L.D. and S.E.P.B.; Software, D.L.C., T.E.J. and L.D.; Supervision, T.E.J., D.W.J. and S.E.P.B.; Validation, S.E.P.B.; Writing—Original draft, A.N.H.N.; Writing—Review and Editing, A.N.H.N., D.L.C., T.E.J., D.W.J., L.D. and S.E.P.B

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# SUPPLEMENTARY INFORMATION FOR

## Sensory and chemical drivers of wine consumers' preference for a new Shiraz wine product containing *Ganoderma lucidum* extract as a novel ingredient

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**Table S1.** Basic chemical composition of the *GL* wines

| Treatment samples | pH | TA (g/L) | Ethanol (% v/v) | VA (g/L) | Free $SO_2$ (mg/L) | Total $SO_2$ (mg/L) | Color (AU) | Sugar (g/L) |
|---|---|---|---|---|---|---|---|---|
| CONTROL | 3.91 cd | 4.56 ab | 12.75 c | 0.25 c | 48.53 c | 123.73 b | 10.54 f | 0.61 f |
| PRE 1 | 3.90 d | 4.21 d | 12.3 d | 0.25 c | 51.73 b | 117.33 c | 11.67 e | 0.74 e |
| PRE 2 | 3.92 c | 4.29 cd | 13.16 b | 0.26 c | 52.8 b | 125.87 b | 11.83 d | 0.98 d |
| PRE 4 | 3.96 a | 4.48 b | 12.76 c | 0.29 b | 58.66 a | 133.87 a | 12.56 c | 1.56 b |
| POST 1 | 3.94 b | 4.63 a | 13.83 a | 0.34 a | 36.26 d | 90.67 e | 12.59 b | 1.32 c |
| POST 4 | 3.97 a | 4.35 c | 13.48 ab | 0.3 b | 36.26 d | 96.53 d | 14.62 a | 2.96 a |

Data are means of triplicate measurements, except VA measurements were conducted in duplicate. Means within a column followed by different letters are significantly different (one-way ANOVA, Tukey's HSD, post-hoc, $p < 0.05$). Abbreviation: Titratable acidity (TA), volatile acidity (VA).

**Table S2.** Mean intensity ratings of sensory attributes significantly differentiated six wines from RATA sensory test.

| Sensory attributes | CONTROL | PRE 1 | PRE 2 | PRE 4 | POST 1 | POST 4 |
|---|---|---|---|---|---|---|
| Ap-red | 4.31 a | 3.75 c | 4.11 ab | 3.72 c | 3.91 bc | 3.91 bc |
| Ap-brown | 2.11 d | 2.35 cd | 2.60 bc | 3.68 a | 2.91 b | 3.91 a |
| A-red fruit | 3.86 a | 3.45 ab | 3.43 ab | 2.57 c | 2.95 bc | 3.05 bc |
| A-dried fruit | 1.55 c | 1.54 c | 1.98 abc | 2.20 ab | 1.75 bc | 2.34 a |
| A-confectionery | 2.83 a | 2.89 a | 2.65 ab | 2.25 b | 2.42 ab | 2.15 b |
| A-cooked vegetable | 0.57 ab | 0.25 c | 0.34 bc | 0.78 a | 0.20 c | 0.80 a |
| A-earthy | 0.77 bcd | 0.62 d | 1.05 abc | 1.12 ab | 0.75 cd | 1.28 a |
| A-floral | 2.32 ab | 2.51 a | 1.88 bc | 1.75 c | 1.94 bc | 1.88 bc |
| A-mushroom | 0.52 bc | 0.42 c | 0.78 ab | 0.57 abc | 0.51 bc | 0.89 a |
| A-leather | 0.49 bc | 0.28 c | 0.74 ab | 0.71 ab | 0.51 bc | 0.85 a |
| A-pepper | 0.74 a | 0.31 b | 0.68 a | 0.65 a | 0.34 b | 0.66 a |
| A-savory | 0.68 bc | 0.54 c | 1.09 b | 0.97 b | 0.82 bc | 1.60 a |
| A-toasty | 0.78 b | 0.80 b | 0.94 b | 1.37 a | 0.77 b | 1.34 a |
| A-woody | 0.85 bc | 0.60 c | 1.06 ab | 1.03 ab | 0.77 bc | 1.28 a |
| A-tobacco | 0.55 b | 0.68 b | 0.45 b | 0.72 b | 0.57 b | 1.05 a |
| T-bitter | 3.05 b | 3.17 b | 3.40 ab | 3.77 a | 3.25 b | 3.69 a |
| F-red fruit | 3.55 a | 3.62 a | 3.25 ab | 2.77 c | 3.06 bc | 2.91 bc |
| F-dried fruit | 1.32 c | 1.29 bc | 1.72 a | 1.97 c | 1.54 abc | 2.15 ab |
| F-jammy | 1.97 a | 1.38 bc | 1.78 abc | 1.37 c | 1.85 ab | 1.98 a |
| F-cooked vegetable | 0.46 ab | 0.25 b | 0.35 b | 0.49 ab | 0.22 b | 0.71 a |
| F-earthy | 0.68 bc | 0.49 c | 0.97 ab | 0.71 bc | 0.82 bc | 1.15 a |
| F-floral | 2.23 a | 1.80 ab | 2.00 ab | 1.14 c | 1.54 bc | 1.65 b |
| F-mushroom | 0.31 c | 0.37 bc | 0.65 ab | 0.58 bc | 0.29 c | 0.92 a |
| F-green capsicum | 0.72 a | 0.38 b | 0.63 ab | 0.65 ab | 0.35 b | 0.72 a |
| F-pepper | 0.82 abc | 0.42 d | 0.94 ab | 0.63 bcd | 0.55 cd | 1.00 a |
| F-savory | 0.71 b | 0.65 b | 1.17 a | 1.29 a | 0.69 b | 1.32 a |
| F-spice | 1.46 abc | 1.34 bc | 1.83 a | 1.31 bc | 1.08 c | 1.75 ab |
| M-astringency | 2.57 ab | 2.35 b | 2.85 a | 2.91 a | 2.68 ab | 2.88 a |
| M-smoothness | 3.75 a | 3.40 ab | 3.40 ab | 3.03 b | 3.18 b | 3.43 ab |
| M-roughness | 2.25 bc | 2.14 c | 2.60 ab | 2.72 a | 2.65 a | 2.69 a |
| FL-non-fruit | 3.72 ab | 3.31 c | 3.60 abc | 3.89 a | 3.34 bc | 3.72 ab |

Means within a row followed by different letters are significantly different. Data were collected and analyzed by using mixed model with Fisher's LSD post-hoc tests, with a significance level of $p < 0.05$. Prefixes: A- = aroma attribute; T- = taste; F- = flavor attribute; M- = mouth-feel, Ap- = appearance, FL- = aftertaste (fruit and non-fruit) intensity of different wine treatments. Prefixes PRE = *GL* extracts added prior to fermentation (PRE 1, PRE 2 and PRE 4), POST = *GL* extracts added after fermentation process (POST 1 and POST 4).

# Chapter 5

# Conclusion and future directions

## 5.1 Concluding remarks

*Ganoderma lucidum* is a renowned medicinal mushroom, which has long been used as a crude remedy in oriental and Traditional Chinese Medicine for the promotion of health and maintenance of vitality. In recent times, as consumers have become increasingly aware of the purported benefits of *GL* products, its use worldwide as a dietary supplement or in other products such as teas has boosted the annual sales of products derived from or containing *GL* to an estimated value of US$2.5 billion (Li et al., 2013). When the term "*Ganoderma*" was searched for on SciFinder Scholar, more than 6500 publications were recorded mainly written in Chinese and focused on the biologically active molecules in *GL* (Baby, Johnson, & Govindan, 2015).

Key challenges the Australian wine industry faces are increased competition and a perceived lack of product differentiation both domestically and overseas. Shifting consumer tastes and the rise of non-traditional, new wine markets globally such as in Asia, places a requirement on Australian winemakers to both "stand out" on the market shelf while meeting the needs and preferences of its key and potential markets in order to grow. Consumers are increasingly becoming aware of what they consume and where those ingestible products come from. From 1 July 2017 to 30 June 2018, Australia's total wine exports rose 20% to AU$2.76 billion. Australia's top export market by value in 2017–18 was China, including Hong Kong and Macau (40 per cent of export value) (Wineaustralia, 2018). Taking advantage of Australia's proximity to other Asian countries in the Asia-Pacific region and its clean and green image, an opportunity existed for production of a novel Australian wine product containing *GL* and utilising Shiraz wine grapes, the most widely planted wine grape variety in Australia, which might be well received in these nearby markets. Limited preliminary research has examined sensory and quality aspects of *GL* containing food products. However, consumers' preference

for novel *GL* grape wine products had not been investigated, and this was viewed as a crucial aspect of wine product development for exporting wine producers who may be willing to develop this niche segment in Pacific markets. Furthermore, no studies had investigated the impact of *GL* present in the grape juice on fermentation progression or the volatile chemical components and sensory characteristics of new *GL*-based grape wine products, and how these factors related to consumer preference.

Therefore the research undertaken in this thesis had a number of key aims: (1) to understand consumers' attitudes towards *GL* wine products using consumers from a variety of cultural backgrounds recruited from three target market populations, i.e., China, Vietnam and Australia; (2) to investigate a method to make a novel Shiraz wine that has either *GL* present in the fermentation or added post-fermentation at different concentrations and then profile the basic and volatile chemical composition as well as sensorial characteristics of the new *GL* wine products; and (3) to understand the tastes, aromas, flavours, and composition of the new Australian *GL* wine products that may drive consumers' preference.

### 5.1.1 *A snapshot of Australian and Asian wine consumer interest and opinions about GL grape wine products*

Within the context of the interest paid by consumers towards *GL*-based products, the first component of the current study (Chapter 1) was a general literature review on *GL* from its taxonomy and components to biological activities of its major compounds.

Chapter 2 described the design and dissemination of a questionnaire containing five sections covering; demographics, alcohol consumption behaviour, wine neophobia, general *GL*-based product consumption and statements in terms of wine consumers' self-reported intent and acceptance of the concept of a new Australian wine supplemented with *GL* extract. A total of 412 wine consumers (Chinese, Vietnamese and Australian) participated in this cross-cultural study. The wine neophobia scale (WNS) was developed by our research group in earlier studies to measure reluctance or avoidance of wine consumers to buy and try never before experienced and novel wines, and was considered an applicable assessment tool when defining new wine

concepts such as *GL*-containing grape wine. While previous studies had focused on developing new *GL*-based products to meet the need of the global market, such as *GL* added into traditional Korean Yakju rice wine or Pilsner beer, no research had examined wine consumers' behaviour or acceptance towards new *GL*-based products, and especially *GL* grape wine products in a cross-cultural context. The WNS for wine product development in culturally diverse target markets was used for the first time, and the main objectives addressed by this study were to understand the factors determining why some Asian and Australian consumers purchase functional foods and beverages containing *GL* and the cultural attitudes of wine consumers, from different countries and wine neophobia segments, towards novel wine products (i.e., those not familiar to the consumers) containing *GL*. Consumer insights regarding acceptance and willingness to try were also gained.

The results were promising for a new Australian *GL* containing grape wine, as they revealed that all consumer groups accepted the notion that *GL* wine products would be worth tasting and they would try them at social events, with Vietnamese consumers being particularly interested. Three wine neophobia segments were identified consisting of 110 wine neophiles, 190 wine neutrals and 112 wine neophobes. The study found that Australian and Chinese participants were significantly more wine neophilic than their Vietnamese counterparts. This reflects the current status of the more mature Australian wine drinking market, the rapidly growing grape wine market in China, and the relatively new Vietnamese wine market. Not surprisingly, wine neophiles were more willing to taste and buy *GL* wine products whereas the neophobes and neutrals were not. The study provided the wine industry insights about consumers' attitudes towards a new *GL* wine product targeted to Australian and Asian markets, which may assist in the development of new niche wine categories containing traditional Asian medicine extracts and enhance consumers' satisfaction.

5.1.2 *Exploring the impact of the presence of GL extract on Shiraz wine fermentation and the sensory attributes and chemical composition of a Shiraz grape GL wine product*

The results of Chapter 2 showed that the use of *Ganoderma*-based products was much more common for Chinese and Vietnamese consumers than Australian consumers. However, regardless of nationality, all consumers were accepting of the concept of a new Australian red wine containing *GL*. This was encouraging, should wine producers wish to explore opportunities for unique wine products in these markets. Following on from the positive consumer response, the study in Chapter 3 examined the influence of different *GL* concentrations added prior to or post alcoholic fermentation on the fermentation process and the chemical composition and sensory profiles of new *GL* wine products. Firstly, despite its known anti-microbial activity, the presence of *GL* extracts (up to 36, 9 and 4 g/L) in ferments at varying scales (100 mL, 5 L and 28 L, respectively) did not hamper primary alcoholic fermentation conducted by *Saccharomyces cerevisiae* wine yeast. The larger-scale ferments also underwent successful malolactic fermentation, indicating the lactic acid bacteria were also not impeded by the presence of *GL*. This study also demonstrated that increasing levels of *GL* extract resulted in small but statistically significant differences in wine basic chemical components (pH, titratable and volatile acidity, ethanol, colour and residual sugar) across treatments. Furthermore, ANOVA identified 39 out of 54 sensory attributes that significantly differed between wine samples and Principal Component Analysis (PCA) of the treatment replicates showed that no sensory differences between pre- or post- addition treatments were observed. Depending on extract level, wines had different sensory profiles. Wines made on the largest scale with the highest concentration of *GL* (4 g/L) were more complex, dominated by savoury, woody, toast, tobacco, mushroom, pepper and earthy aromas, pepper, green and mushroom flavours, higher astringency and roughness and bitter taste; whereas wines with the lowest level of *GL* (1 g/L) were described as having more red fruit, floral and confectionery aromas and flavours, smooth mouthfeel and sweet taste. Wines made with 2 g/L *GL* were more herbaceous, with green capsicum, peppery, spicy and jammy notes. Finally, relationships between wine composition and sensory profiles were explored based on quantitative chemical data and RATA sensory results. Eight volatile compounds, including 2-phenylethanol, ethyl

acetate, limonene, decanoic acid, plus 1-octanol, and hexanoic, octanoic, and 3-methylbutanoic acids, were found to differ significantly between the treatments, with the latter four occurring above their respective odour detection thresholds. Interestingly, a clear separation between *GL* wine groups in terms of specific volatiles, which correlated with relevant sensory attributes in wines, e.g., mushroom aromas and 1-octanol; red fruity aromas and octanoic and decanoic acids, was revealed. These findings indicated that the use of *GL* in the winemaking process could generate wines with different sensory profiles that could meet the needs of local markets such as those in Asian countries.

5.1.3 *Relating consumer liking of prototype Australian Shiraz GL wine products to sensory attributes and volatile composition*

After determining the chemical composition and sensory profiles of new *GL* Shiraz wine products, Chapter 4 builds on these findings. Results are presented of a consumer study that explored sensory and volatile chemical factors that drove consumers' acceptance and preference for a subset of six wines containing *GL* based on segments formed on individual *GL* wine liking scores. Consumers (n = 124) recruited at a food market in Adelaide, South Australia, participated in a blind consumer tasting of *GL* wine to gather their hedonic responses and completed a survey similar to that in Chapter 2, but exploring consumer subjective wine knowledge as opposed to wine neophobia, to understand whether consumers' wine knowledge levels impacted on their hedonic *GL* wine score. Regarding individual liking scores for each wine, three hedonic clusters were identified; cluster 1 (C1, n = 41), cluster 2 (C2, n = 37) and cluster 3 (C3, n = 46). Similar to the sentiments of participants in Chapter 2, most consumers in the study of Chapter 4 considered *GL* wine products worthy of trying, and despite their subjective wine knowledge and hedonic liking scores, that at a social gathering, they would be willing to try new *GL* wine products.

Sensory attributes of the subset of *GL* wine products were profiled with a RATA panel (n = 65). The study examined the 31 sensory attributes that significantly differentiate the six wines with PCA with the hedonic clusters overlaid. There was a clear separation between hedonic

clusters. C1 preferred red appearance, pepper, red fruit, cooked vegetable, earthy, mushroom, leather and woody aromas, and pepper, green capsicum, spice, cooked vegetable, mushroom and floral flavours, and more astringent mouthfeel in wines with 2 g/L *GL* added pre-fermentation (PRE 2) and Control. In contrast, C2 liked wines that were browner in appearance and with more tobacco aromas made with 1 g/L (PRE 1 and POST 1) and 4 g/L *GL* added post-fermentation (POST 4). C3 liked wines made with 1 g/L *GL* and Control wines that had floral and confectionery aromas and red-fruit notes with a smooth mouthfeel.

Since eight volatile chemicals also significantly differentiated the *GL* wines, partial least squares regression analysis was conducted to determine the significant sensory and volatile flavour compounds that were important positive drivers of liking among the hedonic clusters. Drivers were considered the most important when the regression coefficients with absolute values were higher than 0.2. The majority of sensory attributes and volatile compounds did not predominantly influence the overall liking across the three hedonic clusters, as regression coefficients were < 0.1. However, positive liking drivers of C1 were octanoic acid, green capsicum and spice flavours, plus non-fruit flavour aftertaste which, along with hexanoic acid, negatively drove liking of C2. Jammy flavour and tobacco aroma strongly drove C2's preference, while tobacco was important for C1's negative liking. The majority of drivers of C3 liking were related to negative coefficients, including 1-octanol, 3-methylbutanoic acid, hexanoic acid, brown appearance, dried fruit, cooked vegetable and savoury notes, earthy, leather, toasty, woody aromas, bitter taste, mushroom flavour, astringent and rough mouthfeel and non-fruit flavour aftertaste but none of them were considered highly important as the regression coefficients were less than -0.1. These findings provide the wine industry with deeper insights into consumers' liking towards new *GL* wine products targeted at the Australasian market.

## 5.2 Future directions

*GL* is one of the most popular ingredients for foods and beverages with purported functional properties currently available in the Asian market. Compared to conventional wines

in local markets in Asian countries, the notion of *GL* grape wine products, such as those presented in this thesis, is novel. Being a unique wine product, it follows that this work provides the basis for a wide range of research questions for future studies with respect to wine production, economic aspects such as wine marketing, sales, wine distribution channels, and the potential profitability of new wines in the local and global market.

The results from Chapter 2 are the first to provide information on consumers' acceptance towards the concept of new wines containing *GL* cross-culturally (Australian, Chinese and Vietnamese populations), providing evidence that novel *GL* wine products appear to be a promising product. This should be encouraging for wine producers and exporters who wish to explore the economic potential of novel *GL* wine products, or indeed other wine products containing traditional Asian edible extracts, in Australasian markets. It is recommended however, that further research with larger consumer numbers be conducted in these and other Asian markets. Such studies could explore the values these consumers place on *GL* wine products and identify their product evoked emotional profiles, the latter being a newer consumer measure that is known to provide more information than just liking. This coud permit matching products to consumers' emotional needs. Consumption behaviour and usage data could also be collected. For example, it would be useful to understand the context under which consumers might use these products; at certain occasions, events, celebrations, dinner with family or friends, or as gifts. Furthermore, deeper exploration of other wine styles containing *GL* extracts that may be preferable would be valuable knowledge to enhance the consumer experience of these wines.

The new wine product for this research was made using minimal intervention winemaking processes, by simply using Shiraz red grape juice mixed with different levels of *GL* extract and conducting primary fermentation in inert containers or adding extract to finished wine after primary fermentation. Findings showed that at the smaller, research scales examined, the presence of *GL* between 1 up to 36 g/L did not cause sluggish or stuck fermentations. Reflecting on this and the positive consumer taste test results of Chapter 4, which found that wines made

with 1 g/L were most prefered compared to the other wines, it would be useful to conduct further winemaking trials. Firstly, for wines to be made on larger commercial scale, the practicalities of sourcing the amounts for such additions requires examination and any wine instability issues (both chemically and microbiologically) need to be identified. Furthermore, trials could assess wine products made from different grape varieties, regions, wine shelf-life and production methods, e.g., incorporating grape skins during fermentation or the use of oak contact, as is usual for red winemaking. It is likely these more complex wines could handle higher levels of *GL,* although fining of phenolics may be required or the retention of some residual sugar after fermentation allowed, to remove and/or suppress perceived bitterness. Finally, given that only a small number of volatile compounds and sensory attributes were identified as predictors of wine liking from the PLS analysis in Chapter 4, more extensive profiling of wine volatiles and polyphenolic compounds and other *GL* components, could be examined to see if the predictive models of *GL* wine liking improve.

In summation, this study provided consumer insights regarding a novel Australian Shiraz wine product containing an extract of the highly revered *GL*. The online survey highlighted that this type of product would be acceptable and, independent of nationality, wine consumers would be willing to try a *GL* wine product. This project has also advanced knowledge of the main volatile compounds driving the Shiraz *GL* wine sensory attributes and on the relationship of these volatiles and sensory attributes with wine product production, i.e., either pre- or post-fermentation *GL* additions. This can provide wine producers options to alter the resultant sensory profiles of the wine to target the flavours they desire. Through consumer surveys, central location consumer wine preference tests, and a novel rapid RATA descriptive analysis method for wine using naïve consumers, deep insights, including identification and understanding of different consumer segments' preferences were gained. Specifically, relationships between wine flavour chemistry and sensory attributes underpinning consumer preference were identified, which could guide future product development. On the whole, the consumer-centric, new product development approach incorporating novel rapid wine sensory

methods with consumers presented in this study, could provide useful guidelines for those interested in the development of innovative foods and beverages with *GL* or other extracts.

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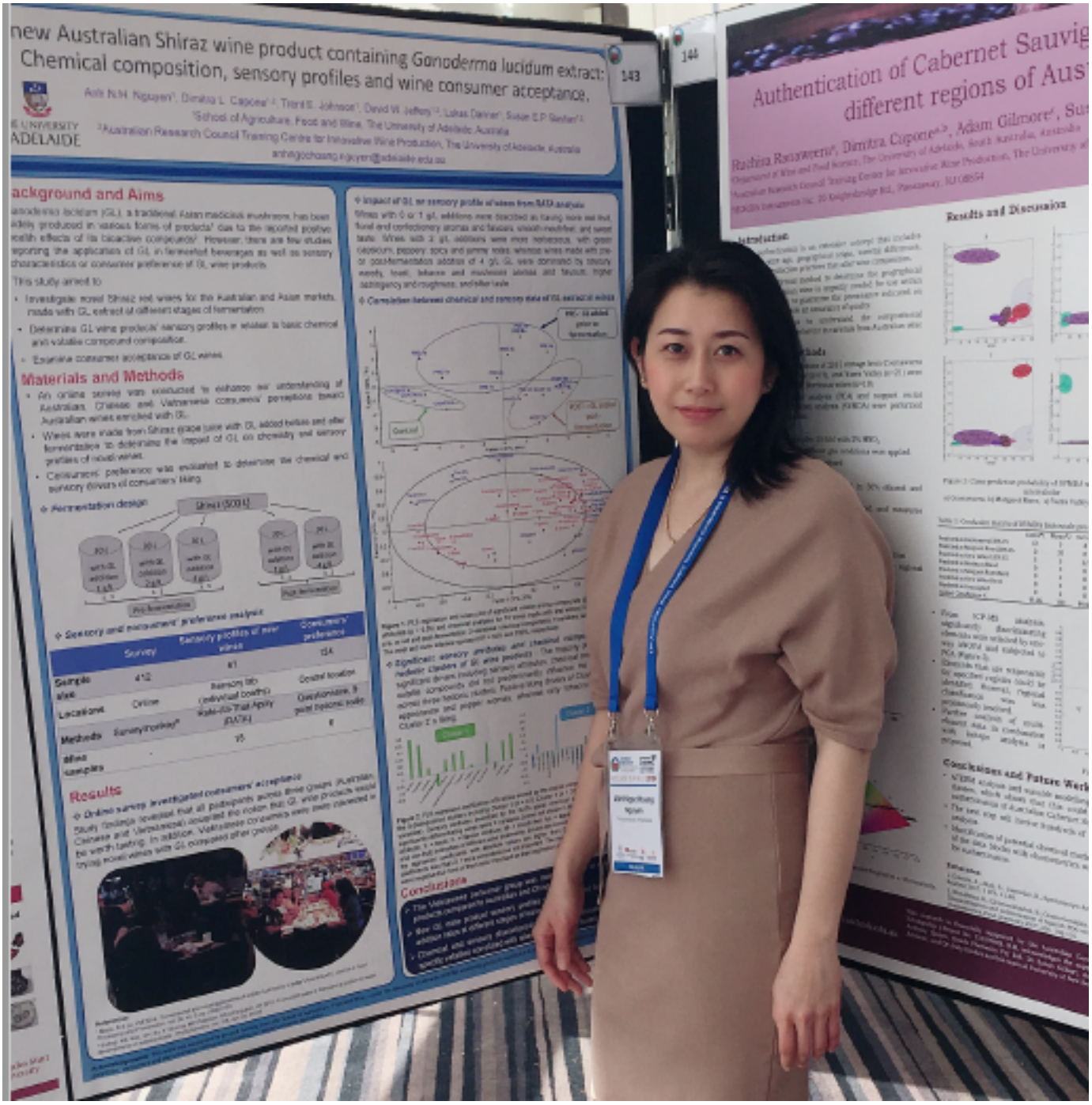
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