

FORMULATION AND PHYSICOCHEMICAL EVALUATION  
OF VINEGARS PRODUCED FROM MURTA (*Ugni molinae*  
TURCZ.) AND MAQUI (*Aristotelia chilensis* (Molina) Stunz)  
WITH BEES' HONEY (*Apis mellifera* L.)  
AT LABORATORY SCALE

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In this study, vinegar was formulated from mixtures of murta (*Ugni molinae* Turcz.) and maqui (*Aristotelia chilensis* (Molina) Stunz) with bees' honey (*Apis mellifera* L.) and its physicochemical characteristics were evaluated with the aim of adding value to these agricultural products and diversifying the supply of gourmet vinegars. Alcoholic fermentation was performed by preparing a must by dissolving honey at 21 °Brix with murta pulp and maqui juice in distilled water, which was enriched with ammonium sulphate and ammonium phosphate. The must was inoculated with *Saccharomyces cerevisiae* and incubated at 26 °C to facilitate the action of the yeast. The alcoholic drink was incubated at ambient temperature with *Acetobacter aceti* bacteria to induce acetic fermentation. The vinegar was clarified, filtered and then stabilised in order to measure total, volatile and fixed acidity, pH, ash and total polyphenols. The results indicated that the physicochemical and functional properties were acceptable and that vinegars made from murta and maqui with honey have market potential.

KEY-WORDS: ALCOHOLIC FERMENTATION; ACETIC FERMENTATION; VOLATILE ACIDITY; POLYPHENOLS; VINEGAR.

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## 1 INTRODUCTION

Vinegar is a consumable liquid which has formed part of human food since the very remote past (SUÁREZ and IÑIGO, 2004). It is widely consumed and is available in all countries and in different varieties (MAZZA and MUROOKA, 2009). It is used principally as a dressing for salads, and also for pickling some meats and vegetables (MADIGAN, MARTINKO and PARKER, 2004). Vinegar is most frequently produced from wine, but it can also be made from substrates such as cider, malt, fruit (apples, pears, grapes, oranges and pineapples), honey, syrups, cereals, rice, hydrolysed starches and even diluted acetic acid (NATERA *et al.*, 2003; BAMFORTH, 2005; SOLIERI and GIUDICI, 2009).

Because these alcoholic liquids are not distilled, they maintain subtle flavours and aromas from their raw materials, which give their vinegars widely-appreciated characteristics (CABALLERO, 2003). Thus, vinegars made from fresh fruits acquire higher sensorial and nutritive qualities (MONSPART-SÉNYI, 2006), because the flavour of these vinegars is influenced by the chemical compounds of the fruits during fermentation (MING, DONG and LI, 2010). This can add further value to the vinegar, since some of the micro-organisms present in the process produce compounds and vitamins which can maintain the health-giving components of the fruit (RASPOR and GORANOVIC, 2008). At the same time, they present a great number of nutritional components in the form of organic acids, amino acids and mineral substances, which play an important role in physiological functions (WANG, 2006).

The consumption of berries has generally been shown to have a positive impact on human health; in the case of *Ugni molinae* Turcz. and *Aristotelia chilensis* (Molina) Stunz, these species have a rich and diverse composition with health-promoting bioactive compounds such as phenolic compounds and vitamin C (SCHRECKINGER *et al.*, 2010; RUBILAR *et al.*, 2011). Both these shrubs are native to southern Chile, and have aroused great interest due to the market value of their berries, which have a pleasant flavour and aroma (TABOADA *et al.*, 2010). The astringent, blackish-purple berries of the maqui are also useful as a natural colourant, due to the presence of anthocyanin pigments (ESCRIBANO-BAILÓN *et al.*, 2006).

From a sensorial point of view, one of the most important characteristics of vinegars is their aromatic impact (DURÁN *et al.*, 2007); murta and maqui have good organoleptic characteristics, such as colour, as well as clear, unique flavours (MING, DONG & LI, 2010). Due to this, and the growing market for fruit vinegars as health food products (OU and CHANG, 2009), there has been a trend towards the development of new products in order to broaden the range of vinegars available on the market.

The objective of this research was to formulate and carry out a physicochemical evaluation of vinegars made from murta and maqui blended with honey as an alternative for adding value to these raw materials and diversifying the supply of gourmet vinegars in Chile.

## 2 MATERIAL AND METHODS

The study was carried out in the Bromatology Laboratory of the Agronomy School of Universidad Católica de Temuco, Chile.

### 2.1 RAW MATERIAL

The raw material for the murta vinegar (*U. molinae*) was murta pulp, which was prepared from selected fresh fruit. For the maqui vinegar (*A. chilensis*), maqui juice was used. Both products were blended with multi-flower bees' honey from Southern Chile. For the alcoholic fermentation, a

pure culture of *Saccharomyces cerevisiae* Meyen was used in a liquid suspension of water-honey 21 °Brix, with an average concentration  $1.05 \times 10^7$  cm<sup>3</sup> of yeasts counted in a Neubauer chamber. For the acetic fermentation, *Acetobacter aceti* bacteria from unpasteurised alcohol vinegar were used (strong vinegar).

## 2.2 SUBSTRATE FOR ALCOHOLIC FERMENTATION

The murta must and maqui juice were prepared by liquidising the fruit and then filtering it to eliminate the seeds. The murta pulp and honey, in equal parts, were mixed with distilled water to obtain a concentration of soluble solids of 21 °Brix. In the case of the maqui, 640 g of honey were dissolved in 1 L of maqui juice; this was then mixed with distilled water to reach 21 °Brix, which was determined with a refractometer. To activate fermentation, 0.2 g L<sup>-1</sup> of ammonium sulphate and 0.02 g L<sup>-1</sup> of ammonium phosphate (ILHA *et al.*, 2000) were added; the amounts added to the maqui were 0.4 g L<sup>-1</sup> of ammonium sulphate and 0.05 g L<sup>-1</sup> of ammonium phosphate. Once a homogenous mixture had been obtained it was placed in glass bottles and then autoclaved for 15 to 20 min at 121 °C. When the temperature of the enriched must fell to 26 °C, it was inoculated with 100 mL L<sup>-1</sup> of the *S. cerevisiae* culture. To encourage alcoholic fermentation, the bottles were incubated in an oven for 2 days at 26 °C. The pH (3.38) and alcoholic percentage (10 %) were measured.

## 2.3 ACETIC FERMENTATION

Once the alcohol content in the murta must reached 8 % (v/v), which was determined by an alcoholmeter, 0.1 g L<sup>-1</sup> of ammonium sulphate, 0.5 g L<sup>-1</sup> of ammonium phosphate, 0.1 g L<sup>-1</sup> of potassium citrate and 0.1 g L<sup>-1</sup> of magnesium sulphate (ILHA *et al.*, 2000) were added to the alcoholic drink. Quantities of 1 g L<sup>-1</sup> of ammonium phosphate; 0.2 g L<sup>-1</sup> of ammonium sulphate and 0.2 g L<sup>-1</sup> of magnesium sulphate were added to the maqui with gentle stirring. Both musts were then inoculated with acetic acid bacteria (*A. aceti*) and fermented at room temperature for one week with oxygenation provided by an oxygen pump.

After both vinegars had been pre-filtered, the murta vinegar was clarified with 90 g hL<sup>-1</sup> of bentonite because it presented turbidity (HIDALGO, 2002). This product favours rapid decanting without altering the composition of the vinegar. Finally, both vinegars were filtered with a vacuum pump using N° 5B filter paper, producing a liquid free of impurities and with a sufficient degree of brilliance.

To prevent *A. aceti* from re-starting the fermentation process, producing turbidity in the bottom of the bottle, or a membrane on the surface, the vinegar was stabilised by pasteurisation in the bottle: the bottled vinegar was immersed in water at 70 °C for sufficient time to reach a temperature of 66 °C during 5 minutes. After this process the vinegar is in its final stage and can be stored at room temperature.

## 2.4 DETERMINATION OF TOTAL ACIDITY

Total acidity was determined using neutralisation volumetry in the presence of an alcoholic solution of phenolphthalein as an indicator, based on the method described by PANREAC (1999). A quantity of 10 mL of previously filtered vinegar was measured in a 250 mL Erlenmeyer flask. It was diluted with 100 mL of cold, recently boiled, distilled water and a weakly coloured solution was obtained. Six drops of the phenolphthalein indicator solution were added and it was titrated with sodium hydroxide 0.5 N, stirring until the indicator turned. The total acidity or percentage acetic degree of the vinegar was determined using Equation 1:

$$\text{Total acidity (\%)} = a \times 10 \times 0.0300 \quad (1)$$

Where: "a" corresponds to the volume in mL of sodium hydroxide 0.5 N.

## 2.5 DETERMINATION OF FIXED ACIDITY

A quantity of 10 mL of previously filtered vinegar was placed in a porcelain capsule and evaporated completely in a water bath. A quantity of 10 mL of recently boiled distilled water was added and it was again evaporated completely. This operation was repeated five times. A quantity of 180 mL of cold, recently boiled distilled water was added; then six drops of phenolphthalein indicator solution were added and it was titrated with sodium hydroxide 0.1 M, stirring until the indicator turned (PANREAC, 1999). The value of the fixed acidity of the vinegar, expressed in grams of acetic acid per 100 mL, was calculated using Equation 2:

$$\text{Fixed acidity} = a \times 10 \times 0.006 \quad (2)$$

Where: "a" corresponds to the volume in mL of sodium hydroxide 0.1 M.

## 2.6 DETERMINATION OF VOLATILE ACIDITY

Following the method described by PANREAC (1999), the value of the volatile acidity of the vinegar, expressed in grams of acetic acid per 100 mL, was calculated using Equation 3:

$$\text{Volatile acidity} = A_t - A_f \quad (3)$$

Where: "A<sub>t</sub>" corresponds to total acidity or acetic degree, expressed in grams of acetic acid per 100 mL, and "A<sub>f</sub>" to the fixed acidity of the same sample of vinegar expressed in grams of acetic acid per 100 mL.

## 2.7 DETERMINATION OF pH

The pH of the vinegar was measured using a potentiometer (Thomas Scientific, model TS-625) previously calibrated with buffer solutions of pH 4 and pH 7 (VILLA and AGUILAR, 2005).

## 2.8 DETERMINATION OF ASH

The ash content was determined following the method described by PANREAC (1999). A quantity of 20 mL of vinegar was placed in a capsule which had been weighed empty in an analytical scale, and evaporated carefully in a water bath to a syrupy consistency. Heating was continued in a moderate sand bath for half an hour. The capsule was placed in a muffle at 550 °C. After 5 min of complete carbonisation, it was removed from the muffle and allowed to cool, and 5 mL of distilled water was added. This was evaporated in a water bath, and the capsule was again placed in the muffle at 550 °C. After it had been allowed to cool in a desiccator, the capsule was weighed with the ash. Equation 4 was used to calculate the ash content expressed in g L<sup>-1</sup>:

$$\text{Ash} = 50 \times P \quad (4)$$

Where: "P" corresponds to the weight in g of the ash content of 20 mL of vinegar.

## 2.9 DETERMINATION OF TOTAL POLYPHENOLS

The total polyphenol content was determined using the Folin-Ciocalteu method described by Georgé *et al.* (2005), using folic acid as the standard, and the results were expressed in mg equivalents of gallic acid per 100 mL of vinegar.

## 2.10 STATISTICAL ANALYSIS

The results of the chemical analysis of the vinegar samples were analysed by descriptive statistics (average three repetitions) (MICROSOFT CO., 2007).

## 3 RESULTS AND DISCUSSION

Table 1 shows the results of the physicochemical analyses of the two vinegar formulations.

### 3.1 TOTAL ACIDITY

According to Article 48 of Decree N° 78 of Chilean Law N° 18.455 (MINISTERIO DE AGRICULTURA, 2010), which fixes norms regarding the production, preparation and commercialisation of ethyl alcohols, alcoholic drinks and vinegars, vinegars in general must comply with a minimum acetic acid content of 4 g 100 mL<sup>-1</sup> in their composition. The vinegars made of murta and maqui with honey therefore complied with current Chilean legislation and the results obtained were within the ranges of total acidity for fruit vinegar determined by Chang, Hsiu-Chin and Shau-Mei (2005) and Dogaru *et al.* (2006). However, although acetic acid is the principal ingredient of vinegar, the total acidity is not a fundamental element for characterising vinegars (ZHANG *et al.*, 2006). For this reason, the addition of honey in the preparation of murta and maqui vinegars gave them a definite, discriminatory value as compared to other groups of vinegars (pineapple, rice, orange), since honey vinegars do not present signs of ethanol in their final preparation, distinguishing them from other vinegars (BOFFO *et al.*, 2009).

**TABLE 1 - RESULTS OF PHYSICOCHEMICAL ANALYSES OF VINEGARS MADE FROM MURTA (*U. molinae*) AND MAQUI (*A. chilensis*) WITH HONEY**

Parameter	Murta	Maqui
Total acidity (g 100 mL <sup>-1</sup> )	4.68	5.02
Fixed acidity (g 100 mL <sup>-1</sup> )	0.29	0.17
Volatile acidity (g 100 mL <sup>-1</sup> )	4.39	4.85
pH	2.71	2.70
Ash (g L <sup>-1</sup> )	0.89	2.05
Total polyphenols (mg eq. gallic acid 100 mL <sup>-1</sup> )	11.74	123.70

### 3.2 FIXED ACIDITY

The fixed acidity determined for vinegars made from murta and maqui with honey presented values lower than those reported in wine vinegars (SÁIZ-ABAJO, GONZÁLEZ-SÁIZ and PIZARRO,

2006). However, the results were within the normal market parameters and complied with Chilean legislation (MINISTERIO DE AGRICULTURA, 2010).

### 3.3 VOLATILE ACIDITY

The values for volatile acidity determined for the vinegars in this study were within the ranges reported in fruit vinegars (PEIXOTO *et al.*, 2010); however, they were lower than those reported by Sáiz-Abajo, González-Sáiz and Pizarro (2006), who obtained volatile acidity values of 5.97 to 6.13 g 100 mL<sup>-1</sup> when evaluating quality parameters in wine vinegars, probably due to the loss of acidity from the bubble-sweeping during oxygenation in acetic fermentation, because this parameter varies with the type and time of fermentation (KOCHER and DHILLON, 2013). Nevertheless, the final product obtained with these native berries presented good organoleptic characteristics, with the colour and flavour characteristic of these fruits. These compensate for the acrid odour of the volatile acidity of vinegars (HIDALGO *et al.*, 2010), which are principally due to acetates formed during alcoholic fermentation (CALLEJÓN *et al.*, 2008) and which have a positive influence on the fruity aroma of the compounds (SU and CHIEN, 2010; MARRUFO-CURTIDO *et al.*, 2012), contributing both flavour and aroma (ZHANG *et al.*, 2006).

### 3.4 pH

The vinegars made of murta and maqui with honey were within the normal ranges reported in fruit vinegars (CHANG, HSIU-CHIN and SHAU-MEI, 2005; PEIXOTO *et al.*, 2010) and native vinegars (pH 2.2) (DI GIROLAMO, D'AMATO and RIGHETTI, 2011). However, the production process used to prepare vinegars should be considered because, depending on the technological process, there may be a risk of incomplete acetification (HIDALGO *et al.*, 2010).

### 3.5 ASH

According to Article 48 of Decree N° 78 of Chilean Law N° 18.455 (MINISTERIO DE AGRICULTURA, 2010), the ash content in vinegar cannot be less than 1 g L<sup>-1</sup>. Vinegar made of murta with honey would not comply with this regulation and would be outside the range for wine vinegars (SÁIZ-ABAJO, GONZÁLEZ-SÁIZ and PIZARRO, 2004; 2006). However, the vinegar made from maqui with honey would comply with the Chilean regulation and the ranges presented by Sáiz-Abajo, González-Sáiz and Pizarro (2004) and Peixoto *et al.* (2010).

### 3.6 TOTAL POLYPHENOLS

According to a study by Peixoto *et al.* (2010), the honey content in the formulation of vinegar contributes an increase of around 50 % to the total polyphenol content. This is because fermentation increases the extraction of phenols (SACCHI, BISSON and ADAMS, 2005), while other phenols form during fermentation (PLATA *et al.*, 2003). However, the source of the honey used must be considered, since the composition of honeys varies widely, depending on the species of bee which produces it, the floral and geographical origin, the harvest and climatic conditions (PEREIRA, QUEIROZ and DE FIGUEIREDO, 2003; QUEIROZ *et al.*, 2007). The same occurs with maqui and murta berries, where the genotype is the principal factor affecting the polyphenol content (FREDES *et al.*, 2012), together with the different climatic conditions where the plants grow (GAMBELLI and SANTARONI, 2004; REYES-CARMONA *et al.*, 2005; ROBBINS *et al.*, 2005; SHENE *et al.*, 2009).

The difference in the quantity of phenolic compounds in red and white wines is not only due to the presence of anthocyanins, but also to the manufacturing process by which the wine is obtained; this would partly explain the values obtained for the vinegar made from murta with honey,

since it contains few anthocyanin pigments, and presents a colour which would put it in the category of white wine vinegars. On the other hand, the vinegar made from maqui with honey presented a higher polyphenol content, since the berries have a significantly higher polyphenol content and antioxidant capacity than other Chilean berries (FREDES, 2009), as well as being rich in anthocyanins (ESCRIBANO-BAILÓN *et al.*, 2006). This agrees with Pinsirodom, Rungcharoen and Liymminful (2010), who mention that in general, samples of darker coloured vinegars tend to present a greater total polyphenol content and antioxidant capacity, due to the raw materials used.

Another possible reason why the vinegar made from murta and honey presented values below the range for total polyphenols compared to other vinegars (NATERA *et al.*, 2003; DÁVALOS, BARTOLOMÉ and GÓMEZ-CORDOVÉS, 2005) might be the manner in which the latter are stored. According to Tesfaye *et al.* (2002), the value of this parameter increases in all vinegars during ageing because a large quantity of phenolic compounds is extracted from the barrel. However, the contact of the vinegars with wood is not very significant for their polyphenol content, because the presence of these compounds is principally due to the raw material used in production, as in the case of wine vinegars, where these have a greater influence (ALONSO *et al.*, 2004). Despite this, the vinegar made from maqui with honey was within the ranges reported by Kocher and Dhillon (2013), who obtained values between 81.9 and 138.6 mg 100 mL<sup>-1</sup> total phenols in sugar cane vinegar, and presented higher values than those reported by Peixoto *et al.* (2010) in vinegar made of orange mixed with honey (43.27 mg 100 mL<sup>-1</sup>).

Thus, both these native berries have a potential for use in vinegar production, with added value for this product based on their polyphenol content, colour and aroma, since consumers are interested in obtaining health benefits from foods and demand products with new characteristics. The principal raw materials for such products are fruits and vegetables (UBEDA *et al.*, 2011), and their most attractive aspect is the consumption of natural antioxidants (especially phenolic compounds) and health-promoting substances (DÁVALOS, BARTOLOMÉ and GÓMEZ-CORDOVÉS, 2005).

#### 4 CONCLUSION

It was possible to produce vinegars from murta and maqui with honey, using commercial yeasts and acetic bacteria as inocula under laboratory conditions. Vinegar production is an alternative for adding value to these three raw materials.

Although none of the levels for the physicochemical properties of vinegar made from murta with honey was notably higher than found in the literature, all were within the normal range and complied with the law, except the ash content, which in the case of murta vinegar was below the level required by Chilean legislation. Under chemical analysis, the vinegar made from maqui with honey presented acceptable characteristics in all the evaluated parameters, thus complying with Chilean legislation.

#### RESUMO

##### **FORMULAÇÃO E AVALIAÇÃO FÍSICO-QUÍMICA DE VINAGRES DE MURTA (*Ugni molinae* TURCZ.) E MAQUI (*Aristotelia chilensis* (MOLINA) STUNZ) COM MEL DE ABELHAS (*Apis mellifera* L.) EM ESCALA DE LABORATÓRIO**

Avaliaram-se as características físico-químicas de vinagres obtidos a partir da mistura de murta (*Ugni molinae* Turcz.) e maqui (*Aristotelia chilensis* (Molina) Stunz), adicionados com mel de abelhas (*Apis mellifera* L.), visando agregar valor à esses produtos agrícolas e diversificar a oferta de vinagres gourmet. Preparou-se o mosto para a fermentação alcoólica, dissolvendo a polpa de murta, o suco de maqui e o mel em água destilada até se obter concentração de sólidos solúveis de 21 °Brix. Visando o enriquecimento do mosto acrescentou-se sulfato de amônio e fosfato de amônio. O mosto foi inoculado com *Saccharomyces cerevisiae* e incubado a 26 °C para facilitar a ação das leveduras. A bebida alcoólica resultante desse processo foi incubada à temperatura ambiente com bactérias *Acetobacter aceti* para induzir o processo de fermentação acética. O vinagre foi

clarificado, filtrado e depois estabilizado, sendo submetido às determinações da acidez total, fixa e volátil, pH, cinzas e polifenóis totais. De acordo com os resultados das análises o produto apresentou propriedades físico-químicas e funcionais adequadas, indicando perspectivas de mercado para o vinagre elaborado com maqui, murta e mel.

**PALAVRAS-CHAVE:** VINAGRE; FERMENTAÇÃO ALCOÓLICA; FERMENTAÇÃO ACÉTICA; ACIDEZ VOLÁTIL; POLIFENOIS.

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