

## Hydromel, food fortification potential

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### Abstract

Quality benefits in current diet led to discovery of medicine-edibles with target functions, associated to food supplements. A new, fast growing market with diverse opportunities for food processors. The biotechnological, natural recovery of honey can be structured in two directions: 1. *hydromel* (stun or wine, depending on the period of fermentation); 2. *vinegar*. The paper proposes the obtaining at lab scale and afterwards the characterisation of hydromel (natural liquid prepared, energising/filled with vitamins/antioxidant), to which herbs have been integrated in the original recipe (mint, cinnamon, thyme, ginger). This approach started from the preventive- functional association with benefits for health. The paper aims at increasing functionality of the product through addition of herbs with healing potential, has been raised. These aspects will be quantified through comparison of some quality characters.

**Keywords:** fortified/functional foods, hydromel, aromatic plants, quality indicators

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### 1. Introduction

Currently, in order to cope with "food stress" as an effect of globalisation, agri-food productive structures are looking for alternatives to advanced processing, by diversifying food supply based on the identification and exploitation of (by)products of natural origin, quantified by the quality and bioavailability of nutritional principles. This trend has as substrate the evaluation of structural, functional, and systemic manifestations associated with diet, determining factors in the rediscovery of some products from the food history. This, initially, generated the emergence/development of the food-medicine segment, *fortified-functional* with target functions, which propose to meet the current nutritional needs of the body, a concept that can be achieved through interdisciplinary collaboration of the research/innovation/application sectors of food engineering/biological sciences. Understanding the mechanisms of action of functionally recognized species as beneficial to health can *customize the "target"* function with the breakdown of the occurrence of conditions.

The development of this product segment involves the identification of the functional component(s) ("*markers*") with health and/or risk prevention benefits for a particular condition or group of conditions with the same aetiology, which may give rise to predictions in the evolution of chronic diseases. In most cases, *markers* are an important source to create a *nutritional-functional decision network*, based on a series of tests (serological, metabolites, coprological and urological). Currently we encounter terms that define *functional-fortifying products*, developed from *natural* ones, as follows: **1.** functional foods; **2.** nutraceutical foods; **3.** pharmaceutical foods; **4.** designer food; **5.** vitafood [1-4]. A *functional-fortifying* food may have a matrix similar to that of conventional food, consumed as part of the common diet, but with a beneficial physiological intake and/or reduced risk of chronic diseases [5, 6]. Solid/liquid preparations containing bioactive compounds ( $\alpha$ -carotene/ $\beta$ -carotene, lycopene,  $\beta$ -glucan,  $\omega$ -3/ $\omega$ -6, anthocyanidins, flavones, tannins, etc.) of different purity can be prepared.

The *protective role* of natural compounds is a niche field of food science and engineering leading to the development of the concept of *molecular nutrition*, aiming to optimize, model and recommend customized diets structured according to metabolic, genetic, and stress factors [7].

In this food class, we find *honey*, a natural, “green” product due to its beneficial/therapeutic potential in the amelioration/treatment of respiratory, gastrointestinal diseases, inflammatory processes, antimicrobial agent. *At present, efforts are being made to bring to the attention of the community alternative, viable applications for the development of functional-fortifying products derived from honey.* One alternative could be *hydromel* (archaic – mead; (Gk *oinos* = wine and L *meli* = honey; E. *medd*, “meath/meathe”, “medu”), a low-alcohol fermented natural beverage. It is mainly obtained by the natural fermentation of three basic ingredients: honey, water and endogenous yeast and/or in combination with natural vegetable products, resulting in different assortments. Result of fermentation of honey must (biotechnological recovery), with the help of yeast cultures, with a final content of 8÷20% (v/v) ethanol, at a temperature of 10÷18°C, to which fruit, spices, cereals, hops can be added. Archaeological sources attest to the consumption of the product before wine and beer (6500-7000 BC (northern China), present in the Slavic peoples, Greeks, Gauls, Celts, Egyptians, considered a “cure for aging” [23, 24]. Qualification is similar to wine, depending on the amount of sugar: dry (0÷10 g/L), half-dry (10÷20 g/L), semi-sweet (20÷40 g/L), and sweet (40-60 g/L) [25]. Fermentation rate depends on the nature of the honey, the yeast strain, the nutrient support, and the pH of the environment. Finally, one gets a natural drink with a sweet-sour, slightly acidic taste. The assortment of honey, the yeast, the additive elements, the maturation process (“aging”), *imprints the character and defines hydromel* (colour, taste, aroma, bouquet). It is recommended to use *natural, fresh, unfermented honey* to obtain a quality finished product [8-10]. The exogenous intake of (*beer*) yeast is optional, but it has some advantages: **1.** it reduces the fermentation time (*a parameter pursued in this paper*); **2.** it intensifies/accelerates the fermentation process; **3.** it produces low amounts of acetaldehyde; **4.** it results in complete consumption of carbohydrates with the accumulation of low volumes of SO<sub>2</sub>.

Hydromel can be obtained by various methods: **1.** *Godan* (with grape yeasts); **2.** *Derosne and Layens* (with bee bread); **3.** *Cabas and Ware* (with wine yeast); **4.** *Jacquemine* (selective ferments) [26]. Depending on the production method, hydromel is classified as follows: **a.** pepper; **b.** cider; **c.** melomel; **d.** meteglin. The ratio of honey: water (fruit juice) (v/v), may be different: 1:0.5; 1:1; 1:2 and 1:3 [11]. The share of honey depends on: **1.** the predictive amount of must; **2.** the alcohol content; **3.** the type of product (dry, sweet, etc.). It is known that, to obtain 1° of alcohol, 17 g/L of sugar is needed and, in the case of honey, knowing that ≈70% of sugars are fermentable, 24.5 g/L is needed for the same result. Problems occur in the *fermentation stage* when the increase in acidity induces the production of volatile esters, altering compounds (especially taste and aroma) [12]. *Fermentation* is influenced by a number of factors: **1.** *temperature* (at 10°C, the process is very slow or incomplete; at 32÷36°C, yeasts are destroyed); **2.** *oxygen* (although the process is anaerobic, yeast needs oxygen to multiply); **3.** *alcohol* (antiseptic action; above a certain limit, it causes yeast inactivation); **4.** *minerals* (nutritional support for yeasts). The *alcoholic fermentation* of the must goes through three phases: **1.** *pre-fermentation* (CO<sub>2</sub> release; impurities appear; the temperature increases up to 17÷18°C; carbohydrates, must density and volume are reduced (≈5÷12% of the initial volume); duration of 1÷3 days); **2.** *tumultuous* (temperature reaches 25÷30°C; the share of sugars and density decreases; alcohol content and the amount of CO<sub>2</sub> increase; duration of 5÷14 days); **3.** *post-fermentation* (= quiet fermentation) (reaching the ambient temperature; clarifying the must; defining the character). *Raw hydromel* can be *clarified* with bentonine, gelatine, or filtered, followed by the maturation period (1÷10 years), in which aromatic compounds develop, especially ethyl acetate (= it develops, in time, a solvent-like odour), depending on the acetic acid content [13-15]. The beneficial/fortifying imprint is given by over 400 bioactive compounds (vitamins (C, A, D, K, B complex), minerals/trace elements (potassium, calcium, magnesium, phosphorus, selenium, chromium, iodine, etc.), biological water). The benefits of consuming hydromel are: it is vitaminizing; it supplies natural enzymes and hormones; it is a nerve tonic; it is a vasodilator; it promotes the secretion of gastric juice; it prevents cardiac ischemia, inflammatory processes, bacterial

processes, insomnia, degenerative diseases, etc. [27]. It is interesting the correlation of studies of the climate, soil vegetation and vegetation factors, which allows to know their relations with the crop with their requirements to environmental conditions. Based on these was initiated and perfected crops ecological zoning, into natural conditions according with biological requirements of plants to them [16]. The first documentary attestation of hydromel in the Romanian space dates from 1413, when the merchants from Brasov pay tax for its sale [28].

## 2. Materials and Methods

### 2.1. Materials

2.1.1. Primary: **1.** water (from the water supply system); **2.** poly-floral honey (western Romania) (action: antioxidant; it stimulates appetite; it prevents bone demineralization; it is tonic; it prevents/treats anorexia in children; it increases the percentage of haemoglobin in the blood; it is digestive; it improves heart and liver activity); **3.** raw pollen; **4.** alcoholic propolis extract (tincture) (wide therapeutic applicability); **5.** yeast (*Saccharomyces cerevisiae*) (exogenous intake was intended to reduce the fermentation time  $\Rightarrow$  acceleration of biochemical reactions).

2.1.2 Auxiliary (from the commercial network): **1.** mint (*Mentha piperita* L.) (action: antiemetic, it stimulates the secretion and elimination of bile (flavonoid compounds), it is anti-fermentative, disinfectant (tannins), spasmolytic, anticancer (menthol), antiseptic, analgesic, anti-inflammatory); **2.** cinnamon (*Cinnamomum verum*) (action: healing, antiulcer, cytoprotective, antiseptic, anti-infective, antibacterial (destroys over 96% of pathogenic bacteria), antiviral, antifungal (*Candida*, *Aspergillus*), antiparasitic, antispasmodic, antispasmodic, haemostatic, invigorating/tonic; recommended in neuropsychiatric disorders)); **3.** thyme (*Thymus serpyllum* L.) (action: diuretic, choleric, digestive, anthelmintic, antiseptic, nerve tonic); **4.** ginger (*Zingiber officinale*) (action: antiemetic, anti-vertigo, carminative, digestive stimulant, cough reliever, analgesic, sedative and antipyretic) [17].

### 2.2. Methods

2.2.1. The classic, artisanal, manufacturing method was chosen as follows:

- in an open recipient with a volume of 6L initially mix 1 kg poly-floral honey/200 g pollen/20 mL

propolis tincture/5 g yeast (previously dissolved in 50 mL water) (*Saccharomyces cerevisiae* – marketed)/5 L the water;

- 5 samples of equal volumes (1L) were constituted, in glass recipients with a volume of 2L provided with fermentation cap (= fermentation I); this formula was chosen due to the tumultuous fermentation (**fermentation I**), exothermic, with CO<sub>2</sub> release; duration 5 days, conditioned by exogenous yeast intake; temperature 20°C, the result is raw hydromel (must) (**H**) (Fig. 1);



Figure 1. Primary fermentation phase

- at the end of primary fermentation, after repeated trials, the following formula of mass of vegetable material (A2) was agreed: constant dry mass (8 g) for ginger (**GH**), cinnamon (**CH**), thyme (**TH**), except for mint (4 g) (**MH**), fresh;
- reformulated/fortified samples (+ control sample (**H**)) (Fig. 2a (from left to right: **H**, **GH**, **MH**, **TH**, **CH**), were maintained for 10 days (= **fermentation II**), at environmental temperature; a gravitational separation action was observed 5 hours after incorporation, for the fortified samples with formation of phases of different solid/liquid densities; the most intense was observed in the **TH** sample followed by **CH**, **MH** and **GH** (Fig. 2b); the end of the secondary fermentation interval was signalled by the absence of the release of carbon dioxide, the deposition of yeast and other substances (pollen, precipitated proteins) at the basis of the containers;
- the initial clarification trials (separation on filter paper coupled with vacuum pump (Fig. 3a) did not give results, probably because of the characteristics of the solid phase (concentration, quantity, temperature, granulation, agglomeration tendency, etc.) and of the liquid phase (viscosity, density, electrolyte concentration, etc.); subsequently, the resulting filtrate was stored (12 h), under refrigeration conditions at 4°C, resulting in a more accentuated clarification of the liquid fraction (*supernatant*) (Fig. 3.b);

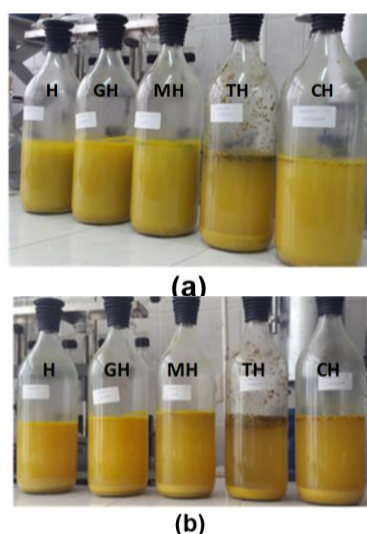
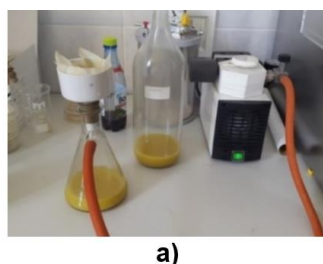
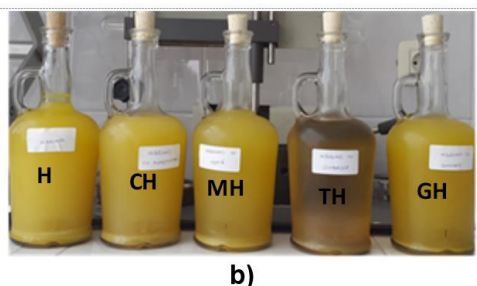


Figure 2. Fortified hydromel: a) in the first hour; b) at 5 o'clock.



a)



b)

Figure 3. Preliminary clarification: a) vacuum pump; b) after 12 hours in refrigeration conditions

- the result led, in addition, to the access of the centrifugal fractionation, corroborated with temperature monitoring in refrigeration conditions (Fig. 4a,b) (RT6000 Refrigerated Bench Top Centrifuge, Sorvall (Fig. 4a)); container capacity: 8x50 mL; operating parameters:  $t^{\circ}\text{C}$  ((- 20 ÷ (+ 40)), speed (0÷6000 rpm), time (0÷35 min); after preliminary trials, the operating parameters were chosen in which the time (15 min), the speed (3400 rpm) and the temperature (4°C) were constant; the action of the centrifugal force, corroborated with the temperature that modifies the rheological behaviour, generated two distinct phases: 1. a solid, opaque, adherent layer, at the lower part of the container (solid phase); 2. a

liquid layer, fluid at the top (supernatant) of characteristic colour (Figure 6b); the filtrate was collected in glass containers (Fig. 4b), stored refrigerated (4°C), subsequently characterized;



a)



b)

Figure 4. Centrifugal clarification: a) apparatus; b) result.

2.2.2. The previous description led to the elaboration of an operating flow diagram (Fig. 5).

2.2.3. Comparative evaluations: 1. microbiological (fungi and coliform species) [18]; 2. antioxidant activity (CUPRAC method) [19]; 3. total polyphenols (Folin-Ciocalteu method, photometric dosage) [19]; 4. sugar content, refractometric method [°Brix] [20]; 5. relative density [21]; 6. free acidity [22]. Statistical analysis: all determinations were made in triplicate and the results are reported as mean values  $\pm$  standard deviation (SD). Differences between means were analyzed with a one-way ANOVA, followed by multiple comparison analysis using the t-test (two-sample assuming equal variances). Differences were considered significant when  $p$ -values < 0.05. All the statistical analysis was performed using Microsoft Excel 2010.

### 3. Results and Discussion

**Microbiological and antimicrobial analysis.** The samples insaminated were maintained for 72 hours at a temperature of 25°C, followed by reading and interpreting the results. The microbiological analysis confirms the safety of the product, which, together with the other determined characters, recommends the product for consumption.

The absence of filamentous fungi was found in all 5 samples analysed after inoculation in the *Sabouraud* medium. The high load of yeast fungi (over 300 germs/mL/sample) is in accordance with the composition of the product.

The presence of yeast stimulates the fermentation of sugars, resulting in alcohol and carbon dioxide.

Another advantage is given by the limiting effect on the development of filamentous fungi that could contaminate the product and direct the fermentation towards by-products of alteration of the physical-chemical and organoleptic characters. Simultaneously with the determination of the fungi, the microbial load with coliform germs of the hydromel was determined by the incorporation technique, ascertaining the absence of the coliform species (Table 1).

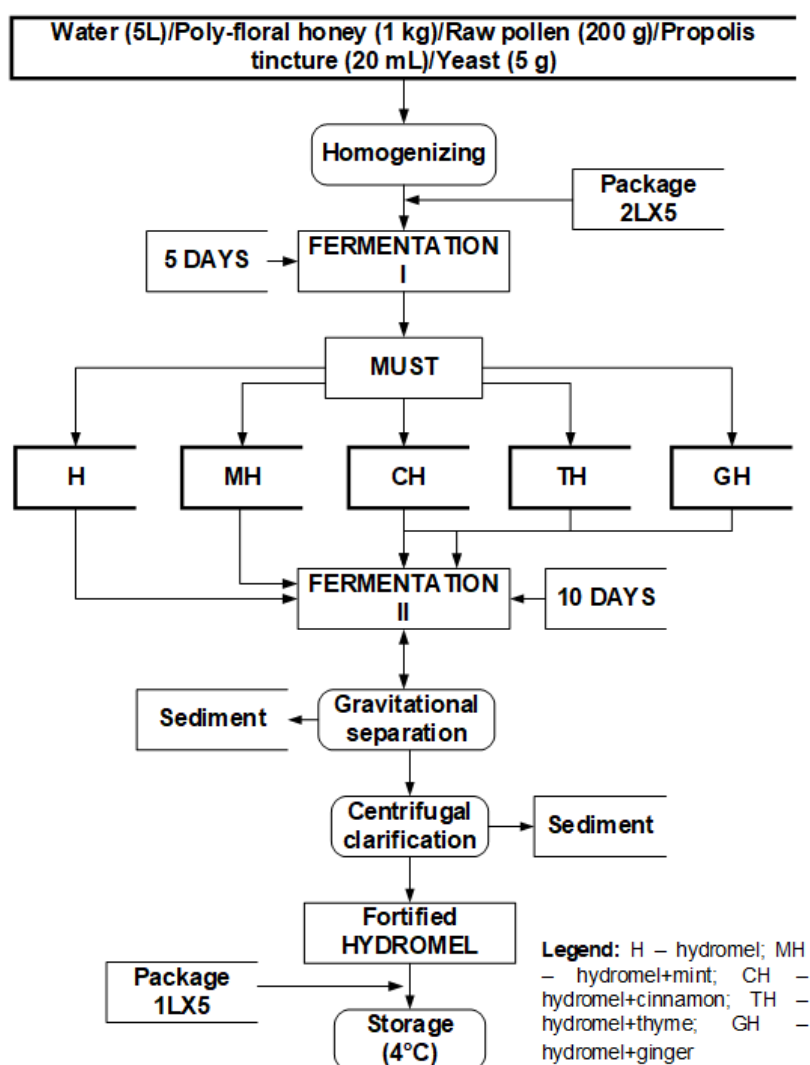


Figure 5. Proposed manufacturing flow diagram for fortified hydromel

Table 1. Comparative evolution of the coliform load

Source	Microbiological load, [germs/mL/sample]		
	<i>Escherichia coli</i>	<i>Salmonella</i>	<i>Enterobacter</i>
H	absent	absent	absent
HM	absent	absent	absent
CH	absent	absent	absent
TH	absent	absent	absent
GH	absent	absent	absent

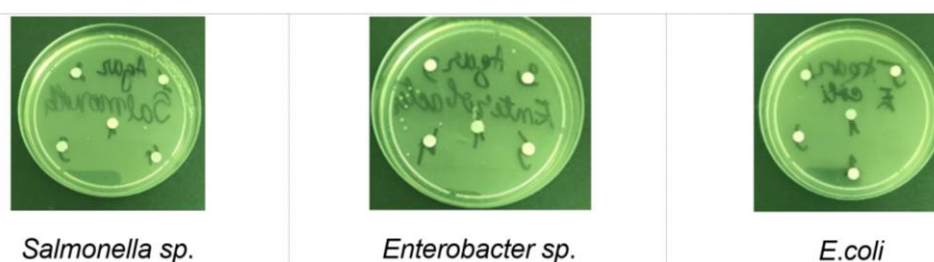


Figure 6. Aspect of antibacterial activity for the strains analysed in the presence of the five product types.

Simultaneously with the determination of the fungal load, the antibacterial activity was evaluated (Fig. 6) in the five assortments obtained, exclusively on coliform germs, and the results were different. Thus, Fig. 6 presents the results obtained using as sample microorganisms – *Salmonella* sp., *Enterobacter* (*Enterococcus faecalis*) and *Escherichia coli*.

Monitoring the microbial colonies in the presence of micro-compresses soaked with different types of hydromel, it was found that, in the case of bacteria belonging to the species *E. coli*, all five types of hydromel inhibit its development. For *Salmonella* and *Enterobacter*, a bacteriostatic activity is observed in the case of **HM** (2) and **GH** (5) samples, the *control sample* (**H** (1)) *does not inhibit* the development of these two bacteria, the colonies developing even in the vicinity of the micro-compressed extract

Table 2. Evolution of antioxidant capacity.

Source	Antioxidant capacity [mg/L]
<b>H</b>	7259.772
<b>HM</b>	1862.542
<b>CH</b>	4802.502
<b>TH</b>	6834.152
<b>GH</b>	1971.754

Evolution of antioxidant capacity (different letter between samples indicate significant differences ( $p < 0.05$ ) between values according to the t test)

**Antioxidant activity** (CUPRAC method) (Table 2). Due to this property conferred by honey, hydromel can provide the body with protection against negative reactions with the formation of free radicals. Currently, studies in the field are intense, promoting and demonstrating the prophylactic beneficial offer against degenerative diseases. A maximum of the activity is recorded for the unfortified sample (**H**) (7259.772 mg/L), with the highest content of polyphenols, and the lowest activity observed for the sample with mint (**HM**) (1862.542 mg/L), correlated with the lowest polyphenolic content (1.252  $\mu\text{moles/mL}$ ).

It can be stated that the share of polyphenols contributes to the potentiation of antioxidant mechanisms.

**Total polyphenol content** (Folin-Ciocalteu method) (Table 3). Antioxidants *par excellence*, polyphenols are associated with protective, beneficial, coronary effects and against chronic diseases. Studies in the field have attributed bioactivity and anti-inflammatory/anti-tumour efficacy to the “fortifying” of the cells under the “invasion” of free radicals (antioxidant action). They are water-soluble products extracted in the primary fermentation phase. They contribute to the formation and definition of the sensory characteristics of the product (colour, aroma, astringency). Analysing the values in Table 3 and their graphical evolution, a decrease of the share of polyphenols in the fortified samples was observed compared to the raw sample (**H**) (1.398  $\mu\text{mol/mL}$ ). The minimum was recorded for the **MH** sample (1.252  $\text{mol/mL}$ ). This shows, contrary to expectations, that the intake of polyphenols by incorporating vegetables with fortifying potency does not accumulate in the finished product and there may be biochemical, physical-chemical causes that reduce/consume a certain quantity of polyphenols. Therefore, further research is needed in this direction. Results show that the antioxidant activity of the products is closely related to the evolution of the total polyphenol content. Therefore, the conclusion is that the antioxidant activity is also influenced by the behaviour and reactions of the elements depending on the physical and chemical stimuli of the operations that participate in obtaining the finished product (fermentation / clarification / storage).

**Sugar content** [ $^{\circ}\text{Brix}$ ] (Table 4). Graphical evolution highlights a slight increase and uniformity of the sugar content in the fortified samples. The minimum (6.5) one was recorded in sample **H**.

**Relative density** (specific gravity) (Table 5). The graphical evolution of the relative densities for the analysed samples is relatively constant after going through the two separation stages (gravitational and centrifugal filtration). This finding further confirms that the operating parameters of the separation/sedimentation operation are decisive in ensuring the efficiency of the process and in the crystallization of elements induced by refrigeration temperatures. The size of this indicator can be a measure of product quality.

**Free acidity** [%] (Table 6). This parameter falls within normal limits, even slightly alkaline (7.7), for sample **H**, and the lowest (6.2), for the sample with ginger (**GH**) (slightly acidic). These values can also describe the conduct of the fermentation operation (time, temperature), but also the contribution of flavouring materials to the formation of the final characteristics.

**Table 3.** Evolution of polyphenols.

Source	Total polyphenol content [µmol/mL]
<b>H</b>	1.398
<b>HM</b>	1.252
<b>CH</b>	1.323
<b>TH</b>	1.363
<b>GH</b>	1.283

Evolution of polyphenols (different letter between samples indicate significant differences ( $p < 0.05$ ) between values according to the t test)

**Table 4.** Evolution of sugar content.

Source	Sugar content [%Brix]
<b>H</b>	6.5
<b>HM</b>	7.3
<b>CH</b>	7.2
<b>TH</b>	7.5
<b>GH</b>	7.5

Evolution of sugar (different letter between samples indicate significant differences ( $p < 0.05$ ) between values according to the t test)

**Table 5.** Evolution of relative density

Source	Relative density [g/cm <sup>3</sup> ]
<b>H</b>	1.072
<b>HM</b>	1.076
<b>CH</b>	1.085
<b>TH</b>	1.084
<b>GH</b>	1.099

Evolution of relative density (different letter between samples indicate significant differences ( $p < 0.05$ ) between values according to the t test)

**Table 6.** Evolution of free acidity

Source	Free acidity [%]
<b>H</b>	7.7
<b>HM</b>	6.7
<b>CH</b>	6.7
<b>TH</b>	6.3
<b>GH</b>	6.2

Evolution of free acidity (different letter between samples indicate significant differences ( $p < 0.05$ ) between values according to the t test)

#### 4. Conclusions

Research confirms the continuation of the detection of new variants/methods and fortifying materials in enhancing the biologically-active and nutritional value of hydromel as a viable alternative in the direction of therapeutic-nutritional use of honey. It is brought to attention a drink little known, but with potential, derived from the diversification of the raw material, in accordance with the perception of the consumer, who intensely requests new options/products related to the agri-food offer. Data confirm the dependence of the fermentation time on the character and type of honey, of yeast strain and fermentation conditions (must agitation, yeast nutrient support, pH control), which can regulate production time. Due to the nature of the subject approached, the study of hydromel remains an area with great potential due to the quality of the materials and to the method of obtaining which can classify fortified products as *organic* fortifiers. The conclusions formulated have implications on the behaviour and orientation of consumers, on the *modelling/integration* of non-invasive therapies and on the revision of nutritional education practices.

**Compliance with Ethics Requirements.** Authors declare that they respect the journal's ethics requirements. Authors declare that they have no conflict of interest and all procedures involving human or animal subjects (if exist) respect the specific regulation and standards.

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