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Physiological benefits of a honeydew-based functional food fortified with selected bioactive agents justified by trials

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Abstract. Honey is a consumer-preferred, highly esteemed natural product with a broad variety of distinct bioactive components. In recent days, the consumption of high-added-value, honey-based products are increasingly coming to the forefront of interest, and thus huge efforts are being made by researchers/developers to elaborate honey variants with fortified biological value. Relevant human clinical trials have scarcely been accomplished; thus, the biological impact of honey and its derivatives has not been thoroughly revealed. In this work, we present our experiments on the development of a novel honey-based prototype and its plausible physiological impacts certified via human clinical trials. The investigated product was a newly elaborated honeydew-based prototype fortified with pumpkin, sea buckthorn, and inulin, which was subjected to a 13-week-long, double-blind, placebo-controlled human clinical trial. The prototypes were applied to 20 adult volunteers to establish the complex impact of the newly developed product. Conclusions drawn at the end of the trial were based on results of blood tests taken at diverse phases of the study. The positive physiological effects of consumption of the investigated products are underpinned by the fact that no significant elevations have been measured in terms of the blood glucose level and parameters featuring long-term blood sugar levels. Slight decrease of both LDL and HDL cholesterol levels were also experienced.

Keywords and phrases: inulin, honeydew, functional food, pumpkin, sea buckthorn

1. Introduction

In recent days, wellness trends and conscious nutrition are getting more and more into the forefront of interest. The consumption of healthy foodstuffs is not only a fashion currently, but it also turns out to be the conviction of many people as a health-conscious lifestyle. In accordance with changing consumer demands, food manufacturers elaborate complex foodstuffs (functional foods) of certified high added value and health-promoting effects (*Cencic & Chingwaru*, 2010; *Daliu et al.*, 2019; *Mračević et al.*, 2020).

The study presented in this paper aims at revealing the physiological impacts of a newly developed, honey-based functional foodstuff, which is in full compliance with this modern nutrition trend. A unique functional food prototype has been elaborated in the initial phase of the studies: a special blend of inulin-fortified honeydew and plant extracts of high added value.

The nutritional and physiological relevance of various honey types has already been thoroughly studied and characterized (*Siro et al.*, 2008; *Salonen et al.*, 2017). Acacia honey in particular is one of the most favoured honey types in many countries with a fructose:glucose ratio higher than that of other honey types, resulting in less likelihood of crystallization during storage (*Mădaş et al.*, 2019; *Kiss et al.*, 2019).

Honey might be considered as a healthy alternative of any artificial sweetener possessing various kinds of drawbacks in terms of health implications. The glycaemic index of saccharose is 68 while that of acacia honey is just around 50–55 (*Deibert et al.*, 2010; *Sadeghi et al.*, 2019).

The application of inulin in a variety of foodstuffs in order to replace fat or sugar or to modify the sensory characteristics has already been studied (*Tiwari et al.*, 2015). With one-tenth of sucrose sweetness, inulin comprises about 25–35% less energy than digestible carbohydrates and is considered a dietary fibre (*Shoaib et al.*, 2016; *Ahmed & Rashid*, 2019).

The application of inulin in functional foodstuffs is a sensible way of increasing the biological value due to its well-proven prebiotic activity (*Bouhnik et al.*, 2007; *González-Herrera et al.*, 2015). Inulin comprising honey-based products has not been introduced, but an inulin-rich syrup (EAT TROO) has already been developed, which is now commercially available.

Capilano Honey Ltd. has recently launched a new product called "Beeotic Honey", whose beneficial properties are to be justified via clinical tests. This prebiotic honey contains eight naturally occurring and formed oligosaccharides and is listed as a therapeutic good with the Therapeutic Goods Administration. A study conducted by the University of New South Wales (UNSW) revealed that it led to the increase of several of beneficial bacteria and suppressed harmful bacteria in the digestive tract (*Cokcetin*, 2015).

Relevant human clinical studies have been implemented by us in order to justify some of the beneficial features of the new product as well as to reveal its actual physiological impacts.

2. Materials and methods

The new product prototype

In our unique approach, the implication of not only honeydew as a healthy foodstuff but also of probiotic inulin and antioxidant-rich fruits is accomplished. Such a combination of the abovementioned components has not been described before.

The novel food prototype is composed of 84% honeydew, 10% pumpkin, 5% sea buckthorn, and 1% inulin. Several combinations have been tested, and the mentioned composition led to the most appropriate organoleptic traits as well as texture. Our primary intention was to apply the components of marked health-promoting impact in the highest possible concentrations, though the upper limits were determined by the revealed physical-chemical properties, with special regard to the solubility and homogeneity of the solution. It was observed that increasing water content resulted in shorter storage time; sea buckthorn was used as powder.

The elaborated, functional honeydew was subjected to regular quality analyses (water-content, HMF, antioxidant activity) in order to ensure the long-term excellent quality of the product. The developed honeydew-based prototype was not prone to sugar precipitation as opposed to several other honey types.

The daily dose of the product included in the diet of volunteers (chiefly as a breakfast supplement) was 6.6 g, which did not signify a stressful amount for them. The only disadvantage experienced was the product's cragged texture, which can be attributed to the applied sea buckthorn powder. The potential positive impact of our investigated product might derive from its unique composition and the multiple, synergistic effects of the intrinsic components.

Major constituents and their biological relevance

The honeydew

The honeydew honey (forest honey, pine honey) is a product with high sugar content, dark, opaque colour, balsamic aroma and strong taste, produced by bees from the body secretions of plant pests. Although honeydew honey is a special type of honey, it differs from nectar honeys in many nutritional and physiological aspects. It contains protein, vitamins, minerals, volatile compounds, alkaloids, and antioxidants. The slightly lower sugar content can be characterized by a 1:4

fructose/glucose ratio, so we can expect a higher amount of fructose compared to rapeseed and fruit honey. The content of oligosaccharides with potential prebiotic effect is much higher than that of nectar honeys.

Honeydew honey contains higher amounts of glutamic acid and tryptophan than nectar honey. Among the honeys, honeydew has outstanding mineral and essential microelement content (406 mg/100 g in total). The substantial potassium content (239–364 mg/100 g) makes up 74% of the total mineral content.

Honeydew honey is rich in volatile compounds, such as terpenes, which give it a scent. Literature findings revealed that terpenoids can exert antimicrobial, antifungal, antiviral, and anti-inflammatory effects.

Compared to nectar honey, one of the major benefits of honeydew is its three times higher content of polyphenols (140.6 mg/100 g GAE/100 g) and flavonoids, resulting in outstanding antioxidant capacity. As a consequence, liver-protective, antibacterial, antiviral, anti-inflammatory, anti-tumour, and cholesterol-lowering effects might be attributed to honeydew.

The pumpkin

Pumpkins are characterized by high water (91.6%) but low energy (108.8 kJ/100 g), protein (1 g/100 g), and fat content (0.1 g/100 g). Pumpkins may also be involved in the preventive effect against cardiovascular diseases. Most of the carbohydrates – 6.5 g/100 g – in pumpkin are polysaccharides, and just a small proportion is constituted by sugars (2.7 g/100 g).

Pumpkin fibres typically contain water-soluble, highly water-binding pectin. The pectin slows gastric emptying and prolongs the absorption of nutrients, thus increasing the amount of stool and maintaining health. Pectin consumption may also contribute to lowering total and LDL cholesterol levels.

Some pumpkin polysaccharides have both antioxidant and prebiotic effects, so they can have a beneficial effect on blood lipid values and protect against pathogenic bacteria. Out of the carotenoids, it contains beta- and alpha-carotene, lutein, and zeaxanthin in the largest amounts.

The sea buckthorn

Recently, several studies have addressed the medicinal use of some components of sea buckthorn (*Hippophae rhamnoides* L.). Potential utilizations have been demonstrated in the areas of cancer, cardiovascular diseases, gastric ulcer, inflammatory diseases, thrombosis, diabetes, tendon injuries, cholesterol reduction, and antibiotic, antiviral effects. Sea buckthorn fruit is one of the fruits of low sugar content (0.79–6.63 g/100 g).

In addition to fruit pulp, oil-rich (7–11%) sea buckthorn seeds are also present in the investigated product. Sea buckthorn seed oil consists of 35% linolic acid and 34% α-linolenic acid, so in seed oil the presented omega-3/omega-6 fatty acid ratio is nearly 1:1, which is favourable for maintaining health. Sea buckthorn oil is an outstanding source of phytosterols, containing 522–577 mg/100 g of beta-sitosterol.

Sea buckthorn is abundant in antioxidant vitamins such as vitamin C (600 mg/100 g), tocopherols (216–481 mg/100 g), but it also contains higher amounts of folate (80 mg/100 g), vitamins B_1, B_2, and K. The sea buckthorn pulp contains remarkable amounts of vitamin C and iron (4–15 mg/100 g), which can contribute to the reduction of fatigue as well as to the normal functioning of the immune system and the metabolic processes. Sea buckthorn comprises large amounts of phenolic compounds, accounting also for its outstanding antioxidant effect.

Inulin, a powerful prebiotic

The investigated honeydew-based functional product was enriched with pumpkin and sea buckthorn extract and contained 1% inulin. Several beneficial effects of prebiotic inulin consumption have been shown recently such as positive influence on bowel habits, gastric health, and immune system.

Inulin promotes calcium absorption very effectively and is able to modulate the immune function by increasing the SCFA content of the colon. Due to its immunomodulatory effect, inulin may help controlling irritable bowel syndrome. As a plausible component of potential synbiotics, it significantly reduces CRP and increases glutathione levels. In addition to the positive physiological impacts listed above, several more health-promoting effects have been confirmed in previous studies (*Markowiak & Slizewska*, 2017; *Plaza-Díaz et al.*, 2017; *Yu et al.*, 2017; *Huang et al.*, 2018).

Setup and implementation of the human clinical trial

The objective of the study was to gain information on the actual physiological impacts of the novel functional food product, and to find a way to incorporate its consumption into the daily diet of the volunteers as well as future consumers.

The study was accomplished in total compliance with all relevant EFSA requirements as well as with the ethical guidelines of the valid Declaration of Helsinki. Good clinical practice was applied in accordance with CPMP/International Conference on Harmonization ([ICH]/135/95).

The human clinical trial was a 13-week-long, crossover, double-blind, placebo-controlled randomized study with self-control groups. The study period was divided into four phases. The 1st phase included a screening and a baseline visit, where participants were randomly selected and informed of the use and dosing of the

investigated product. Thereafter (2nd phase, or first study period), the participants consumed daily a single dose of honeydew or the investigated product for 5 weeks: 5 ml (6.6 g) of the product once a day in the morning. The 3rd phase was a 3-week-long wash-out period, when the participants did not consume any honey products. Afterwards (4th stage, or second study period), in a 5-week-long period, the volunteers who had consumed functional product in the first study period, were supplied with the control substance, while those who were previously given the control product as a supplement to their food, this time consumed the functional product. This crossover feature of the study made it possible to survey the effects of both products in the case of the same volunteers.

At the beginning of this period, one on-site visit and later a telephone visit was performed, and the closing visit took place at the end of the final week. During the thirteen weeks, the condition of the participants was checked with the examination of general parameters, laboratory tests, and objective questionnaires. The results obtained after the statistical evaluation were analysed from a physiological, dietetical, and technological point of view alike.

Changes in some relevant parameters were monitored with blood tests taken at diverse phases of the study. The following laboratory parameters have been examined regarding the study's aim and by maintaining tolerability and safety: K, Na, Ca, P, Mg, Cl, Fe, total cholesterol, HDL, LDL, triglyceride, AST; ALT; GGT; alkaline phosphatase, bilirubin, HbA1c, CRP, BUN; creatinine; eGFR, albumin; total protein, MCH, MCV, polyphenol content, and antioxidant activity.

Statistical analysis

SPSS 26.0 for Windows (IBM SPSS Statistics, IBM Corporation, Armonk, NY) was used for the statistical analysis of the data. A descriptive analysis was performed for all variables. Normally distributed, continuous data were described as means and standard deviations. Results are provided as mean and its 95% confidence interval plots. The significance of differences among groups was evaluated with one-way analysis of variance (ANOVA) followed by Tukey's comparison test. Categorical data were described as numbers and proportions and analysed with a χ^2 test. The value was statistically significant at the level of $p < 0.05$.

3. Results and discussions

The justification of the physiologically beneficial properties of the new prototype was accomplished in our studies. The major laboratory safety parameters did not display significant alterations, and all the surveyed such parameters remained within the normal interval, indicating that the consumption of the investigated product is safe.

Neither honeydew nor its fortified, functional variant led to elevation of blood sugar level despite their high sugar content. It might be deduced that the daily consumption of 6.6 g of the investigated product might be considered a safe dosage even for people suffering from diabetes. It is also remarkable that parameter HbA1c, being associated with the blood sugar level, exhibited stagnant values. This phenomenon can be regarded as a health benefit of the investigated product despite the high overall sugar content; no blood-sugar-related elevations were observed (*Figures 1–2*).

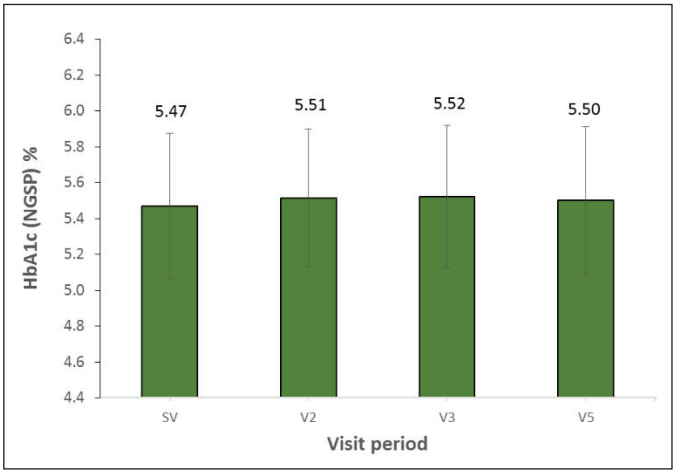

Figure 1. The impact of the consumption of the honeydew-based new prototype on HbA1c (NGSP) level (%)

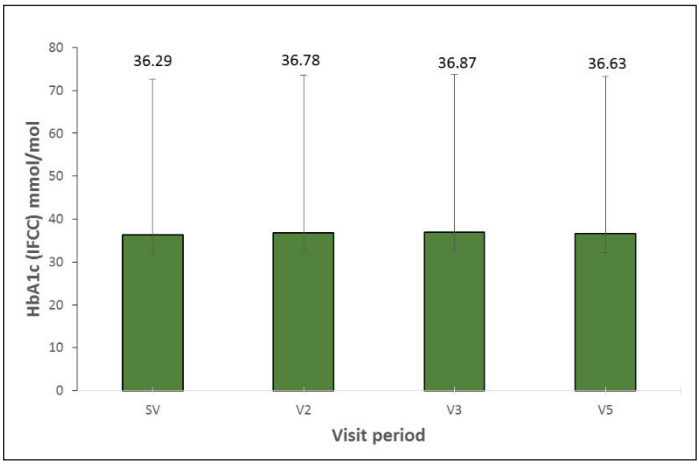

Figure 2. The effect of the honeydew-based new prototype on HbA1c (IFCC) level (mmol/mol)

Thanks to the application of pumpkin and sea buckthorn in the investigated product – being abundant in potassium and iron –, slightly improved K- and Fe-levels were observed upon completion of the study. The corresponding data for potassium are presented below, in *Figure 3*.

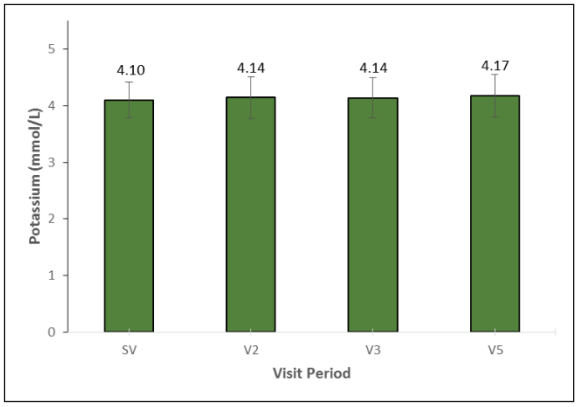

Figure 3. The impact of the consumption of the honeydew-based new prototype on potassium level

The total bilirubin level surveyed during the study exhibited an increasing tendency for the control product and a decreasing trend for the functional product (*Figure 4*). This might be considered as another beneficial consequence of consuming the investigated product, which is ascribed to the high polyphenol content of the included sea buckthorn and pumpkin.

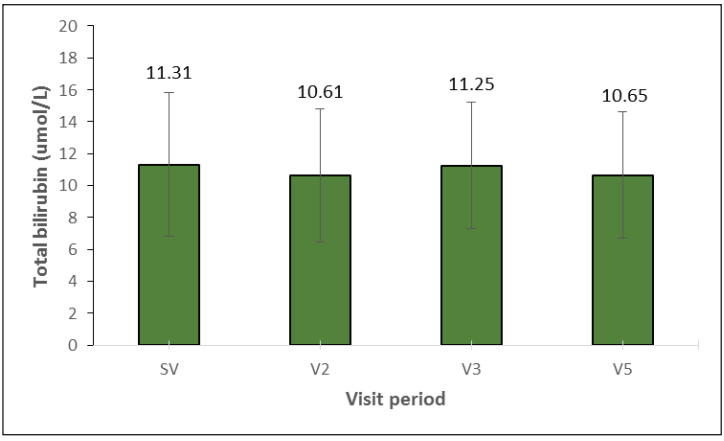

Figure 4. Changes in total bilirubin levels during the study period (13-week-long consumption of honeydew-based new prototype)

The consumption of the investigation product did not result in significant changes in any of the examined lipid parameters. The total cholesterol concentration exhibited a very slight elevation by the end of the study period, which might be accounted for by other nutrition factors apart from the used functional product (*Figure 5*). The beneficial features and high antioxidant activity of the functional product might have hindered the significant elevation of lipid parameters in spite of the fact that the increased sugar input should have triggered a marked increase in terms of these parameters.

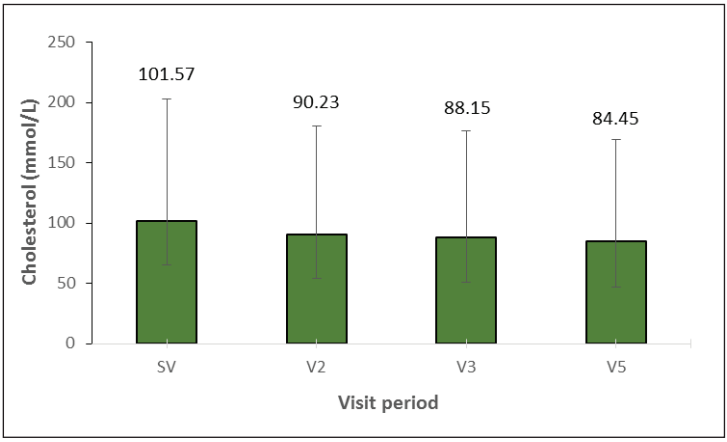

Figure 5. The impact of the consumption of the honeydew-based new prototype on cholesterol level

The consumption of the investigated product did not lead to significant alterations in the HDL cholesterol level; however, a slight decrease was observed upon completion of the study period (*Figure 6*).

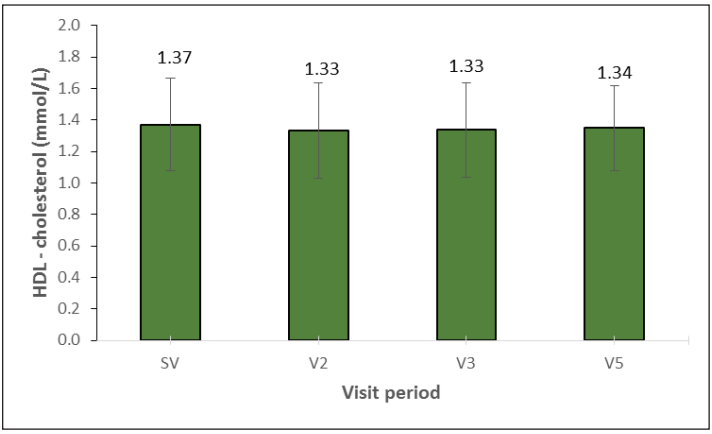

Figure 6. Changes in HDL cholesterol level during the study period

In terms of LDL cholesterol level, no significant changes were observed during the 13-week-long study period; however, a very slight increase could be observed at the last visit (*Figure 7*). This observation did not meet the expectations and might be explained by the consumption of other foodstuffs of unbeneficial impacts.

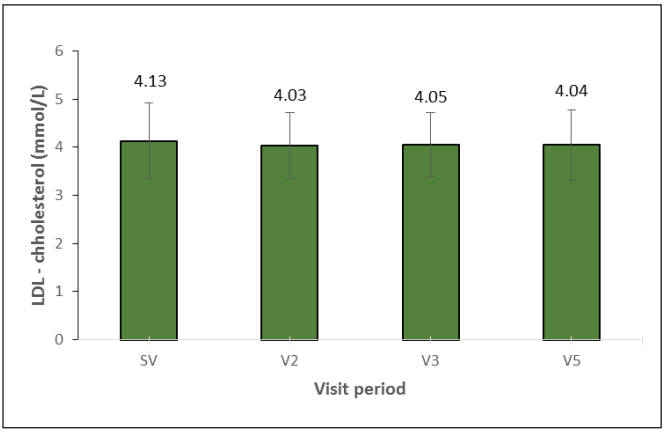

Figure 7. The impact of the consumption of the honeydew-based new prototype on LDL cholesterol level

The alkaline phosphatase activity parameter exhibited a slight decrease throughout the study period as follows: the high polyphenol content of sea buckthorn might have played a crucial role in the development of this tendency. The same parameter of the control product displayed a slight increase throughout the study period, and thus the observed trend can be regarded as a remarkable distinction (*Figure 8*).

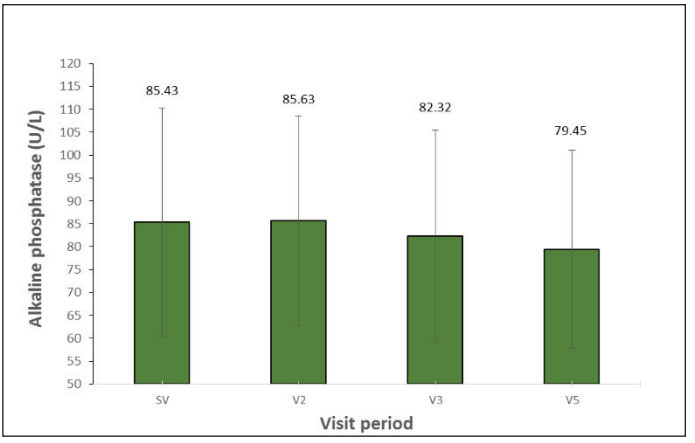

Figure 8. The effect of the honeydew-based new prototype on alkaline phosphatase level

4. Conclusions

In this study, the actual physiological effects triggered by the consumption of a special, newly developed functional foodstuff pointed out that the investigated product's high added value is reinforced.

Most significantly, it should be noted that the new product's long-term consumption is regarded as not only safe but also physiologically beneficial. Multiple synergistic effects of the investigated product's intrinsic bioactive components have been observed. Special emphasis should be laid on the fact that the consumption of the product does not lead to increase in blood sugar; hence, under controlled conditions, even clients with diabetes might be recipients of this food type. HbA1c, the most reliable blood sugar indicator parameter, displayed no increase at all.

Total cholesterol levels displayed negligible changes during the study, which is regarded as a beneficial impact of consumption of the new food prototype. The high antioxidant activity as well the abundance of other types of bioactive molecules present in the investigated product hindered the significant elevation of lipid parameters, which might be regarded as a beneficial consequence.

The new food prototype can be regarded as a healthy alternative for sweeteners due to its relatively low impact on blood sugar level and its high level of diverse, beneficial bioactive substances.

Most of the involved volunteers were impressed by the flavours of our functional product and claimed that they would purchase it in case of commercialization. In the long run, this new product might constitute an integral part of people's regular breakfast meal. The implemented human clinical study facilitated the introduction of the product as well as the elaboration of scientifically grounded marketing activities.

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