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UTILISATION OF QUINOA FOR DEVELOPMENT OF FERMENTED BEVERAGES

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ABSTRACT
Lactic acid fermentation of pseudocereals represents a useful tool for the preparation of value-added beverages with beneficial properties to consumers. The aim of this work was the development of a novel quinoa-based beverage fermented with commercially available probiotic culture including *Bifidobacterium sp.*, *Lactobacillus acidophilus*, and *Streptococcus thermophilus*. The results concluded that fermentation of quinoa beverages significantly increased proteins and total phenolic content and antioxidation activity in the final products (by 36.84%, 26.67%, and 14.74%, respectively). In general, the overall acceptability of unfermented quinoa beverages was low (less than 46%), but the fermentation process slightly increased their acceptability (by 9.43%). A significant improvement of acceptability was observed, when the raspberry syrup was supplemented into the fermented beverages (by 90.98% compared to the no supplemented samples). Viability of fermenting microorganisms, pH, total acidity, and organic acid content were determined during the storage of beverages for 21 days at 5 °C. It was found that prepared quinoa beverages had a good probiotic potential (>6 CFU.mL^{-1} of lactic acid bacteria cocci). Furthermore, this study also showed that the quinoa represents a suitable raw material for formulation novel gluten and dairy-free fermented beverages with increased content of nutritionally important compounds.

Keywords: quinoa; fermentation; lactic acid bacteria; beverages; sensory acceptance

INTRODUCTION
Recently, there is a growing demand for new types of cereal products with a higher nutritional value, which can offer health benefits to consumers due to the content of biologically active substances **(Dallagnol et al., 2013)**. In this respect, considerable attention is focused on the use of pseudocereals, mainly quinoa (*Chenopodium quinoa*) **(Dallagnol et al., 2013; Bhargava, Shukla and Ohri, 2006)**. Quinoa is regarded one of the best vegetal protein sources (12 – 23%), as its protein levels are similar to those found in milk and higher than in true cereals such as wheat, rice and maize **(Gordillo–Bastidas et al., 2016; Kaur and Tanwar, 2016; Nisar et al., 2017; Zannini et al., 2018).** Moreover, quinoa contains a significant amount of starch (52 – 60%) with low amylose content (7 – 11%) **(Ahmed, Thomas and Arfat, 2019)** and the amount of dietary fibre in the quinoa is higher than in the other grains (9 – 16%) **(Gordillo–Bastidas et al., 2016)**. In addition, quinoa is a rich source of bioactive compounds like antioxidants, polyphenols, flavonoids, minerals (magnesium, zinc, iron, potassium, phosphorus) and vitamins (E, B group and C) **(Kaur and Tanwar, 2016; Tang and Tsao, 2017; Ahmed, Thomas and Arfat, 2019)**.

Due to the above mentioned nutritional benefits, the quinoa seeds have recently been incorporated into the functional food products **(Lorruso et al., 2018; Ujiroghene et al. 2019)**. Several authors investigated the potential of quinoa for the production of beverages with using various methods of treatment such as soaking, germinating, cooking, malting **(Pineli et al., 2015; Kaur and Tanwar, 2016; Urquizo et al., 2017; Zannini et al., 2018)**. On the other hand, only a few research works are focused to the production of probiotic quinoa-based fermented beverages **(Bianchi et al., 2014; Lorusso et al., 2018)**. Currently, the study of **Lorusso et al. (2018)** was oriented to assessment the suitability of quinoa for making yogurt-like beverages fermented by using probiotic lactic acid bacteria strains (*Lactobacillus rhamnosus* SP1) and **Bianchi et al. (2014)** developed a potentially synbiotic beverage fermented with *Lactobacillus casei* LC-1 based on aqueous extracts of soy and quinoa with added fructooligosaccharides. More recently **Ujiroghene et al. (2019)** reported that quinoa-based fermented beverages can be applied for diabetes mellitus treatment due to their ability to inhibit α-amylase activity and reduction or prevence hyperglycemic conditions associated with increased levels of sugar glucose in the blood.

Scientific hypothesis

In recent decades, there is an increasing interest of consumers related to the consumption of value-added beverages. In response to consumer demands, this study aimed to evaluate if quinoa could be used for the production of fermented probiotic beverages and could increase its biological value, protein, phenolic content, and antioxidant activity. The sensory acceptability of fermented beverages and their stability during storage was also determined.

MATERIAL AND METHODOLOGY

Raw materials

Quinoa seeds and raspberry syrup purchased from the local Slovak market were used in this study. Before using seeds for the preparation of fermented beverages, the seeds were desaponificated according to the method described by **Urquizo et al. (2017)**. Dried commercial probiotic culture of Lactoflora (Milcom a.s., Prague, Czech Republic) including *Bifidobacterium sp.*, *Lactobacillus acidophilus*, and *Streptococcus thermophilus* was used for the preparation of fermented quinoa beverages.

Preparation of fermented quinoa beverages

Fermented beverages were prepared according to the modified procedure previously documented by **Urquizo et al. (2017)**. Desaponificated quinoa seeds were dried at 60 °C for 8 hours and milled to flour. In the next step, the quinoa flour was mixed with tap water at a concentration of 5% (w/v), afterward, the mixture was gelatinized at 95 °C for 10 min and cooled to 20 °C. The dried commercial probiotic culture was used for direct inoculation of beverage after dilution (initial total lactic acid bacteria count: 10_8 CFU.mL^{-1}). Samples were fermented at 37 °C for 6 hours. After fermentation, the beverages were stored in closed glass containers for 21 days at 5 °C.

Proximate analyses

Proximate analyses of beverages included determination of dry matter (AACC method 44-19.01), proteins by the method of Kjeldahl using a factor of 6.25 (AACC method 46-13.01), crude fat (AACC method 30-25.01) and ash (AACC method 08-01.01) **(AACC, 2000)**. Total dietary fibre (TDF) content was determined by enzymatic-gravimetric method 985.29 **(AOAC, 2003)**. Saponin content in seeds before and after desaponification was measured according to the method described by **Koziol (1991)**. pH of beverages was determined by using a pH-meter (Inolab WTW, Weilheim, Germany). Total acidity was measured by the visual titration method with a standard solution of NaOH (0.1 mol.L^{-1}) and using phenolphthalein as the indicator. Lactic, acetic, and citric acids were determined using the method of capillary isotachophoresis **(Kohajdová Karovičová and Greifová, 2006; Magala et al., 2015)**. Isotachophoretic measurements were realised using an isotachophoretic analyser and the ZKI01 columns connection technique (Villa Labeco, Spišská Nová Ves, Slovak Republic), equipped with a conductivity detector and two-line recorder TZ 4200 (Laboratory instrument, Prague, Czech Republic). The total phenolic content (TPC) was determined using the Folin-Ciocalteu assay **(Park et al., 2017)**. The analysis of TPC was realised by comparison to a calibration curve constructed by gallic acid. Amount of TPC was expressed as milligrams of gallic acid equivalents (GAE) per 100 g of quinoa sample. Antioxidant activity was monitored by DPPH (1,1-Diphenyl-2-picryl-hydrazyl) assay according to the method described by **Kaur and Tanwar (2016)**.

Microbiological analyses

Presumptive counts of lactic acid bacteria cocci and presumptive counts of lactic acid bacteria rods were determined after dilution and cultivation on M17 and MRS agar plates respectively according to STN ISO method 15214 **(STN ISO 15214, 2002)**.

Technological properties

The viscosity of beverages was measured using rotation viscosimeter (Haake VT 550, Haake Mess – Technic, Germany) according to the method reported by **Magala et al. (2015)**. Water holding capacity (WHC) of beverages was determined according to the method previously described by **Zannini et al. (2018)**.

Sensory evaluation

Sensory characteristics of beverages were evaluated by panel assessors (11 – member panel) using a 5 – points hedonic scale (1 – dislike extremely; 2 – dislike slightly; 3 – either like nor dislike; 4 – like slightly; 5 – like extremely) **(Graham, Agbenorhevi and Kpodo, 2017)**. The assessors evaluated overall appearance, taste, odour, colour, and consistency of fermented products. The overall acceptability of beverages was evaluated using 100 mm graphical unstructured line segments with specified end-points **(Kohajdová, Karovičová and Greifová, 2006)**.

In the next step, raspberry syrup sweetened with fructose (selection was performed concerning persons treated to diabetes as potential consumers) was used for increasing sensory acceptability of fermented beverages. After fermentation, the appropriate part of beverages were mixed with raspberry syrup (10%, v/v) and evaluated. The level of this ingredient was chosen on the basis of the previously performed preliminary trial.

Statistical analysis

All analyses were carried out using three independent determinations and expressed as the mean value ± standard deviation. Statistical analyses were performed using Microsoft Excel (Microsoft Corp., Redmond, WA, USA) with XLSTAT for MS Excel Addinsoft SARL, Paris, France). Differences among means were analyzed by Student's *t* - test. The significance level (p) was set at 0.05.

RESULTS AND DISCUSSION

The fermentation of cereals improves the nutritional properties and sensory characteristics of final products and has a positive impact on human health **(Rollán, Gerez and LeBlanc, 2019)**. Nowadays, many scientific studies are focused on the preparation of new types of cereal-based fermented beverages with probiotic and functional properties **(Rathore et al., 2012; Nionelli et al. 2014;**

Ghosh et al. 2015; Magala et al., 2015). Therefore, considering the nutritional and health benefits of quinoa, in this study, the fermented quinoa-based beverages with probiotic potential were developed.

Saponins are bitter compounds that are naturally present in quinoa seeds in the range from 0.1 to 5.0% **(Pytel et al., 2018)**. The content of saponins in the quinoa seeds used in this study was 0.30 ±0.02% (results are not shown). For remove of saponins, washing or water maceration is recommended **(Pineli et al., 2015)**. Washing with tap water was used for removing of saponins in this study. After desaponification, the amount of saponins in quinoa seeds was present in the non-detectable level. Previously **Pytel et al. (2018)** demonstrated a reduction number of lactic acid bacteria during fermentation of yogurts with 3 and 5% content of quinoa flour. Moreover, it was reported that saponins present in the quinoa seeds also cause a negative effect on protein solubility **(Pineli et al., 2015)**. For these reasons, the removal of saponins from the quinoa seeds representing an important step before the production of lactic acid fermented quinoa-based beverages. Proximate composition of quinoa-based beverages before and after 6 h fermentation is presented in Table 1.

During the fermentation process, the pH decreased and total acidity increased significantly in the beverages. This effect was previously described by **Magala et al. (2015)** in rice fermented beverages and **Urquizo et al. (2017)** in the quinoa fermented beverages prepared from various quinoa varieties. A rapid decrease of the pH at the start of fermentation is important to obtain the final product of high quality **(Kohajdová and Karovičová, 2008)**, as well as the rapid increase of total acidity minimizes the growth of undesirable and pathogenic bacteria **(Kohajdová Karovičová and Greifová, 2006)**.

During the lactic acid fermentation, different organic acids (mainly lactic acid) are produced due to the degradation of some components present in the raw material **(Kohajdová, Karovičová and Greifová, 2006; Magala, Kohajdová and Karovičová, 2013; Cho et al., 2015)**. Determination of organic acid profile represents an important tool for monitoring the metabolic activity of fermentation microorganisms **(Cho et al., 2015)**. In this study, the significant increasing of lactic and acetic acids concentration was observed in the fermented beverages in comparison to unfermented samples. Furthermore, slightly decreasing in citric acid content was recorded. This can be attributed to its usage as a substrate in secondary reactions during fermentation process **(Magala, Kohajdová and Karovičová, 2013)**.

Moreover, it was found that, the lactic acid fermentation no significantly influenced dry matter, ash and crude fat content in the quinoa-based beverages. On the other hand, however, after fermentation a significant increasing in protein content was observed in the beverages (increasing about 36.84%). **Tangyu et al. (2019)** reported that fermentation can increase protein content by the growth of the fermenting microorganisms and by improving protein solubility. According to **Simwaka et al. (2017)** the increase in protein content after fermentation has been attributed to the increase in nitrogen content released when microorganisms utilize carbohydrates for energy.

From the results also concluded that the fermentation not significantly influenced the content of TDF in the samples of beverages. Similar trend was also observed by **Marko et al. (2014)** in different fermented cereal matrices. On the other hand, **Jorgensen et al. (2010)** reported that the fermentation of cereals substrates can concluded in reduction of TDF content as a consequence the enzymatic degradation of fibre.

Table 1 Proximate composition, microbial counts and technological properties of quinoa-based beverages.

Fermentation (h)	0	6
Proximate composition		
pH	6.89 ±0.15	4.19 ±0.05*
Total acidity (mmol.L-1)	1.01 ±0.07	5.50 ±0.13*
Lactic acid (g.L-1)	0.25 ±0.01	0.46 ±0.02*
Acetic acid (g.L-1)	0.15 ±0.01	0.26 ±0.01*
Citric acid (g.L-1)	0.22 ±0.01	0.17 ±0.00
Dry matter (%)	5.63 ±0.04	5.83 ±0.02
Ash content (%)	0.04 ±0.00	0.05 ±0.00
Proteins (%)	0.57 ±0.04	0.78 ±0.02*
Crude fat (%)	0.11 ±0.01	0.09 ±0.01
TDF (%)	0.43 ±0.03	0.42 ±0.02
TPC (mg GAE per 100 g of sample)	142.37 ±1.27	180.33 ±1.25*
Antioxidant activity (%)	60.31 ±2.08	69.2 ±1.98*
Microbiological analyses		
Presumptive count of lactic acid bacteria cocci (log CFU.mL^{-1})	6.13 ±0.05	7.21 ±0.08*
Presumptive count of lactic acid bacteria rods (log CFU.mL^{-1})	4.47 ±0.13	6.12 ±0.13*
Technological properties		
Dynamic viscosity (mPa.s)	176.88 ±7.33	236.78 ±6.26*
WHC (%)	74.68 ±2.50	73.90 ±3.32

Note: 0 – quinoa beverages before fermentation, 6 – quinoa beverages after 6h fermentation, TDF – total dietary fiber, TPC – total phenolic contents, GAE – gallic acid equivalents, WHC – water holding capacity, * denotes that means within a line differ significantly (p <0.05).

Table 2 Sensory characteristics of quinoa based beverages.

Fermentation (h)	0	6	6^{x}
	Sensory characteristics		
Overall appearance	2.50 ±0.02	3.31 ±0.04*	4.18 ±0.12*
Taste	2.15 ±0.04	2.77 ±0.06*	4.00 ±0.21*
Odour	2.08 ±0.07	2.46 ±0.09*	4.54 ±0.18*
Colour	3.00 ±0.12	2.88 ±0.10	3.54 ±0.04
Consistency	3.08 ±0.02	2.98 ±0.09	3.10 ±0.09
Overall acceptability (%)	41.67 ±1.03	45.60 ±1.05	79.58 ±2.84*

Note: 0 – quinoa beverages before fermentation, 6 – quinoa beverages after 6h fermentation, 6^{x} – quinoa beverages after 6h fermentation with addition of raspberry syrup, * denotes that means within a line differ significantly (p <0.05).

0 **6** **6^{x}**

Figure 1 Photodocumentation of quinoa-based beverages.
Note: 0 – quinoa beverages before fermentation, 6 – quinoa beverages after 6h fermentation, 6^{x} – quinoa beverages after 6h fermentation with addition of raspberry syrup.

Table 3 Metabolic activity during storage of quinoa based fermented beverages.

Storage (days)	0	7	14	21
pH	4.19 ±0.05	3.99 ±0.04	3.95 ±0.07	3.91 ±0.11
Total acidity (mmol.L^{-1})	5.15 ±0.13	8.00 ±0.21*	10.01 ±0.32*	10.50 ±0.59*
Lactic acid (g.L^{-1})	0.46 ±0.02	0.43 ±0.08	0.48 ±0.01*	0,49 ±0.03*
Acetic acid (g.L^{-1})	0.21 ±0.01	0.19 ±0.03	0.18 ±0.02	0.18 ±0.04
Citric acid (g.L^{-1})	0.17 ±0.00	0.11 ±0.02*	0.03 ±0.00*	ND
Presumptive count of lactic acid bacteria cocci (log CFU.mL^{-1})	7.21 ±0.08	7.13 ±0.16	7.01 ±0.02*	6.12 ±0.08*
Presumptive count of lactic acid bacteria rods (log CFU.mL^{-1})	6.12 ±0.13	5.79 ±0.20*	5.57 ±0.14*	4.94 ±0.01*

Note: * denotes that means within a line differ significantly (p <0.05), ND – non detectable.

Since TDF represent an important functional compound with beneficial health effects, the incorporation of fibre rich raw materials obtained from plant processing by products into the non-dairy beverages can be a good strategy for increasing intake of TDF. In this respect, it seems to be a good alternative application of apple pomace as a fibre rich (more than 50% of TDF) by-product obtained from the juice processing industry that was usefully incorporated to cereal-based products in our previous study **(Kohajdová et al., 2014)**. Moreover, this by product can improve sensory acceptance of non-dairy fermented beverages as a flavouring ingredient due to pleasant fruity odour and taste **(Sudha, Baskaran and Leelavathi, 2007)**.

The phenolic compounds are important for human health and nutrition mainly due to their antioxidant activities **(Hole et al., 2012; Tang and Tsao, 2017)**. As shown in Table 1, the significant increasing of TPC was observed in the quinoa-based fermented beverages. A similar effect was also reported by **Hole et al. (2012)** and **Lorusso et al. (2018)** for the barley, oat, and quinoa substrates processed by lactic acid fermentation. Previously **Bustos et al. (2017)** and **Lorusso et al. (2018)** reported that, during the fermentation process the esterase activity of lactic acid bacteria and endogenous enzymes of cereal substrates can hydrolyse the complex phenolic compounds and their glycosylated forms into free phenolic acids.

Several studies documented that the quinoa seeds are an excellent source of antioxidants and that, the antioxidant

activity is in a good correlation with the content of TPC **(Kaur and Tanwar, 2016; Tang and Tsao, 2017)**. This fact was also confirmed in this study when the increased TPC content in the fermented beverages corresponded to a proportional increase of the antioxidant activity of fermented products.

After 6 h fermentation, the content of lactic acid bacteria cocci and rods reached values 7.21 log CFU.mL^{-1} and 6.12 log CFU.mL^{-1} respectively. The population of lactic acid bacteria in the fermented beverages was increased significantly by 1.08 (cocci) and 1.65 (rods) log orders. This indicates that quinoa is a suitable substrate for lactic acid fermentation.

Consistency represents an important parameter for the development of new functional fermented non-dairy beverages **(Magala et al., 2015)**. The viscosity of quinoa-based beverages significantly increased during the fermentation process. This agreed with **Bianchi et al. (2014)** and **Lorusso et al. (2018)** that also observed increased viscosity in the fermented beverages produced from aqueous extract of quinoa and yogurt-like quinoa beverages. **Ndife et al. (2019)** reported that the increase in the viscosity of cereal beverages after fermentation could be due to an increase in biomass density of the microorganisms.

No significant differences were found in the WHC of beverages before and after fermentation. This indicates that the WHC of beverages remaining stable during the fermentation process.

Sensory characteristics of quinoa-based beverages are shown in Table 2 and photodocumentation of quinoa-based beverages is presented in Figure 1.

Väkeväinen et al. (2020) newly documented that the saponins present in the quinoa seeds decrease the acceptance of quinoa-based products. Following this recommendation and due to the other fact presented in the above part of this article, the quinoa seeds were desaponificated before the use in beverages manufacture. The results concluded that the overall acceptance of nonfermented beverages was lower than 42%. Lactic acid fermentation significantly enhanced overall appearance, taste and odour, but no significantly influenced the overall acceptability of beverages. Fermented beverages were characterised with creamy light colour and sour odour and taste. Due to the low sensory acceptability of beverages, the raspberry syrup was applied as a supplement to improve their acceptability. This ingredient was sweetened with fructose, a sweetener with a low glycemic index that is usually consumed by persons treated to diabetes. The addition of this supplement significantly improved the overall acceptability of fermented beverages. A similar trend was observed by **Urquizo et al. (2017)** after the addition of bilberries and chocolate flavouring to lactic acid fermented quinoa beverages.

The shelf life of fermented beverages was also monitored for 21 days of storage at 5 °C (Table 3). During the fermentation, the pH of beverages slightly decreased. Moreover, the significant increasing of total acidity was recorded. A similar trend was previously described by **Bianchi et al. (2014)** after 28 days of storage of fermented quinoa and soy beverages at 5 °C. Furthermore, it was found that no significant differences were recorded in the content of lactic and acetic acids. The results also concluded that during storage, the citric acid was degraded as the consequence of the metabolic activity of fermenting microflora.

The presumptive count of lactic acid bacteria was also determined in this study. It was observed that lactic acid that presented bacteria cocci and rods showed a high rate of survival percentage during the storage of beverages (84.88 and 80.72% respectively). The viability of bacteria is an important characteristic of the use of probiotics in beverages, once they should survive during the shelf life, with minimal desirable value 6 log CFU.mL^{-1} **(Georgieva et al., 2009; Gallina et al., 2019)**. This value corresponds to ingestion an 8 log CFU per serving portion of 100 mL **(Pereira and Rodrigues, 2012)**. It was found that lactic acid bacteria cocci (*Streptococcus thermophilus*) were present in the beverages at the level above this requirement. On the other hand, the population of lactic acid bacteria rods (*Bifidobacterium* sp., *Lactobacillus acidophilus*) reached at the end of storage only counts 4.94 log CFU.mL^{-1}. **Kurman and Rasic (1991)** and **Samona and Robinson (1994)** previously suggested that the minimum level for probiotic microorganisms in fermented milk to produce therapeutic benefits should be a value above 5 log CFU.mL^{-1}. After 14 days of storage the population of lactic acid bacteria rods remained above this value. From the obtained results can be indicated that experimental beverages had a probiotic character.

CONCLUSION

The potential of quinoa as a raw material for the production of lactic acid fermented beverages was presented in this study. It was shown that the fermentation process significantly increased proteins and total phenolic content and antioxidation activity in the products. Sensory evaluation showed that the overall acceptability of quinoa-based fermented was lower than 46%. The addition of flavouring agent (raspberry syrup) significantly improved acceptability of beverages (79.58%). Regarding these finding, the application of flavourants could be a useful strategy for enhancing consumers' acceptance of these types of beverages. Moreover, further studies are needed to select suitable flavouring agents according to the preferences of potential consumers. The functionality of commercial probiotic culture was demonstrated by high viable bacterial counts in the fermented beverages (7.21 log CFU.mL^{-1} of lactic acid bacteria cocci and 6.12 log CFU.mL^{-1} of lactic acid bacteria rods) and during the storage (above 6 CFU.mL^{-1} of lactic acid bacteria cocci).

In conclusion, the quinoa is naturally gluten-free raw material and thus, quinoa-based fermented beverages represent a suitable alternative for people having celiac disease. Moreover, quinoa has a low glycemic index and in combination with appropriate ingredients, these beverages can be incorporated into the diabetic diet.

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