Formulation, Biochemical Characterization and Shelf-Life Study of YoAlp® Whey Based Beverage Containing Fruits Juices Produced in Aosta Valley

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Article

Formulation, Biochemical Characterization and Shelf-Life Study of YoAlp® Whey Based Beverage Containing Fruits Juices Produced in Aosta Valley

Mathieu Merlet, Tania Flutto, Rita Pramotton, Simona Zenato, Luca Vernetti-Prot and Sabina Valentini *

Institut Agricole Régional – Reg. La Rochère 1/A – 11100 Aosta (Italy); iar@iaraosta.it

* Correspondence: s.valentini@iaraosta.it

Abstract: Background: Functional beverages are ordinary foods with components or ingredients able to provide specific health effects other than purely nutritional effect. **Aim**: This study aims to formulate a new functional beverage obtained from the recovery of YoAlp® whey, a bovine fermented milk obtained using autochthonous starter cultures, added with Renetta Canada/Golden Delicious-Raspberry (YWL), Renetta Canada-Aronia (YWA) and Ravèntse (YWR) fruit juices to improve the functionality of the beverage. **Methods**: Microbiological analysis and chemical characterization, by a proteomic approach and spectrophotometric microassay, have been conducted on beverages to investigate the expected shelf-life and potential health promoting effects. **Results**: The microbiological assessment confirmed the safety of the beverages and the probiotic bacteria vitality over a shelf life of seven days. Biochemical analysis performed highlight the presence of different bioactive peptides (ACE inhibitory and antioxidant), and the betacasomorphin 9 (BCM-9), marker of A2 variant of β-casein typically present in Aosta Valley cattle breeds. Moreover, high amounts of total polyphenols, which concur to the antioxidant activity values observed, have been found. Furthermore, ACE inhibitory activity is well correlated to the bioactive peptides detected. Finally, a consumer test confirmed the YoAlp® whey-based beverages appreciation. **Conclusions**: The production of these beverages could represent a huge opportunity for Aosta Valley's farms to increase their income and competitiveness, creating a circular economy by recovering a by-product like whey and following sustainability and healthy trends, which are driving the food sector.

Keywords: functional beverages; bioactive peptides; polyphenols; probiotic; autochthonous fermented milk

1. Introduction

In the last years, consumers increased their awareness about healthy lifestyle and health promoting foods. Therefore, nowadays foods are not intended only to satisfy hunger and to provide necessary nutrients for humans, but also to prevent nutrition-related diseases and improve consumers' health [1]. These kinds of foods are considered functional foods which are ordinary foods with components or ingredients able to provide specific health effect other than purely nutritional effect. They are not intended as medicines, but they are existing traditional products, intended to be consumed as part of a normal and balanced diet [2].

Among functional foods, dairy-based beverages are very important. Most of them are considered probiotic due to the presence of probiotic microorganisms which interact with the host, or which produce microbial metabolites, and others are enriched with bioactive components such as ω-3 fatty acids, bioactive peptides, Conjugated Linoleic Acid (CLA), vitamins or mineral [2,3]. Frequently, these beverages contain whey as principal component due to its nutritional characteristics. This product is often considered a by-product although its interesting nutritional values. Above all the nutrients, whey proteins are rich in essential amino acids such as lysine,

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methionine and cysteine, and in branched chain amino acids (BCAA) like isoleucine, leucine, and valine [4,5]. Furthermore, between whey protein fractions, there is also lactoferrin, a particular peptide interesting in the production of functional foods due to its beneficial role in consumer's health. This protein has been demonstrated to possess antimicrobic, anti-inflammatory, immunogenic, antioxidant and anticarcinogenic activity [6,7]. Moreover, lactose is the major component of whey, and it represents about 70% of total solids, most of the lactose from the starting milk ends up in whey. In addition, whey is also a good source of electrolytes such as sodium and potassium. Even calcium, magnesium and phosphorus are present in solution and bound to proteins [8]. In addition to these compounds, in these beverages there can be also probiotic microorganisms which are mainly lactic acid bacteria such as *Lactobacillus spp*. and microorganisms belonging to Bifidobacterium genus [9]. They can provide many beneficial effects including improvement of lactose digestibility, anticarcinogenic activity, reduction of serum cholesterol level, synthesis of B vitamins, control of inflammatory bowel diseases, facilitation in calcium absorption and control of pathogenic microorganisms in the intestinal tract [10–14]. To exert these activities, they must be able to survive to the passage through the gut, to proliferate and to colonize the digestive tract. Moreover, they must be safe and effective along the entire shelf life of the product. So as to maintain the effectiveness, probiotic foods must contain over 10E6 UFC/mL live microbial cells at the time of ingestion [15,16].

Along with whey, fruit juices are often used to improve taste and acceptance of consumers and to add important nutrients to the beverage such as vitamins and polyphenols. Consumption of 100% fruit juices can increase the amount of precious nutrients like vitamin A and C, folate, carotenoids, magnesium, and potassium in diet helping to reach the recommended amount of those micronutrients [17]. Among fruit juices those made with Aronia berries are more and more relevant due to a huge antioxidant activity if compared to other berries or fruits. Aronia juice exhibits the highest antioxidant capacity among the polyphenol-rich beverages. In vitro studies show that Aronia has antiproliferative or protective effect against some types of cancers, antimutagenic activity, hepatoprotective effect able to decrease the toxicity and the accumulation of cadmium in the liver and kidney, it decreases serum total LDL cholesterol, and it increases HDL cholesterol, and it has a role in the prevention and control of diabetes mellitus type II and diabetes associated complications [18]. Another important fruit used to produce fruit juice is Ravèntse which is an autochthonous variety of apple cultivated in Aosta Valley that shows high amounts of polyphenols [19].

Considering the relevance of functional foods and problems related to whey disposal, the aim of this project was to create a new functional beverage starting from YoAlp® -whey, a by-product derived from Aosta Valley cattle breed fermented milk obtained with autochthonous strains (*Streptococcus thermophilus* and *Lactobacillus delbrueckii*) inoculation, and fruit juices typically produced in Aosta Valley. Those two ingredients allow to combine many positive and health promoting nutrients such as polyphenols, antioxidants, vitamins, bioactive peptides as well as the natural probiotic lactic acid bacteria. This product could be obtained also by the Aosta Valley's farms to increase their competitiveness and reduce their environmental impact by creating a sustainable process through the recovery of a common by-product.

2. Materials and Methods

2.1. Beverage formulation

Whey has been obtained from YoAlp® straining, a fermented milk developed by Institut Agricole Régional in a project founded by FESR (HEART VdA). YoAlp® has been realized using milk from Aosta Valley's autochthonous breeds and local strains of lactic acid bacteria belonging to species *Streptococcus thermophilus* and *Lactobacillus delbrueckii*. These strains have been isolated during years in small dairies of alpine pastures in Aosta Valley. After the production, to increase the creaminess and concentration of nutrients, YoAlp® has been strained. The strain has been made by gravity suspending the mass in a towel until the 30% of the mass is lost. After that, preparation of beverages has been made by mixing whey with fruit juice in percentages of 60/40%. Fruit juices have been

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produced by local producers, using fruits typically cultivated in Aosta Valley. Three different fruit juices were used: Ravèntse juice, Renetta Canada– Aronia juice and Renetta Canada/Golden Delicious – Raspberry juice. Final beverages have not been pasteurized to preserve lactic acid bacteria, so the shelf life of these beverages has been monitored up to 21 days: 24 hours; 7 days; 14 days and 21 days. The product has been stored in sterilized bottles at refrigerated temperature (4°C).

2.2. Chemical analysis

2.2.1. Total acidity and pH

pH and total acidity for every sample at each shelf-life step were assessed. pH analysis was made through a pH meter. Total acidity was determined by titration with NaOH 0,1 N using phenolphthalein as an indicator.

2.2.2. Peptides

Samples preparation and analysis has been performed according to the method of Sforza et al. 2012 [20] and Rizzello et al. 2005 [21]. Briefly, samples have been centrifuged at 3800 rpm for 30 minutes at 4°C, then filtered with Whatman N° 2 Paper filters. Filtered samples were again centrifuged a 3000 rpm for 1,30 hours with a Cut-off 10 kDa with Amicon Ultra – 2 or 4 – UltraCel 10 K. After that, 5 ml of each sample was aliquoted and evaporated in Centrivap overnight. Evaporated samples were dissolved in 500 µL of mobile phase A and vortexed in a Thermomixer for 90 minutes. Samples were filtered again at 0,45 µm and injected (10 µl) into the column (Jupiter 4u Proteo 90A, 250’4.6 mm). Solvent used during the analysis were mobile phase A (0,2% CH_3CN e 0,1% HCOOH in H_2O v/v) and mobile phase B (0,2% H_2O e 0,1% HCOOH in CH_3CN). These samples have been analyzed by Reverse-phase high performance liquid chromatography coupled with a mass spectrometer (RP-HPLC-ESI (+)/MS). RP-HPLC was performed with ACCELA LC system (Thermofisher Scientific, Milan, Italy) equipped with an auto sampler maintained at 15°C. The output was directly interfaced with an ESI-Ion Trap mass spectrometry LTQ XL (Thermofisher). The positive ion mode has been used, and the mass scan was acquired in a range of 150-1900 m/z. Cone voltage and capillary voltage has been set at 30 V and 3,2 kV. This analysis has been replicated three times per sample. Finally, peptide identification has been performed using an internal database based on literature data and online databases (ESI PROT or BIOPEP). Each peptide has been also associated to a possible bioactive effect and the Peptide Ranker online software gave an indication on the probability of each sequence to exert a bioactive effect.

2.2.3. Polyphenols

Total polyphenolic content was determined by an optimized Folin-Ciocalteu method by Rigo et al. 2000. Briefly, to remove the organic acids, free sugars, free SO_2, amino acids, proteins and other hydrophilic compounds that could cause interference, a preliminary cleanup of the phenols was performed with a Sep-Pak C18 (Waters, Milano, 500 mg) previously conditioned with 2 ml of MeOH followed by 5 ml of 5mM H_2SO_4.Samples were suspended in sulfuric acid 1N with appropriated dilution and analyzed in triplicates. One milliliter of each sample was then slowly loaded on the conditioned Sep-Pak, and the polar substances were removed with 2 ml of 5 mM H_2SO_4. The phenolic compounds were eluted into a 20 ml calibrated flask, with 2 ml of MeOH followed by 5 ml of distilled water. 0.5 milliliter of Folin-Ciocalteu reagent and, after 3 min. 1 ml of 20% Na_2Co_3 were added, and the solution was brought to 20 ml with distilled water. After 20 min at 70°C, samples were filtered at 0.45 µm) and their absorbance was read at 750 nm in a 1 mm cell, against a blank test prepared by using distilled water in place of sample. Concentration was determined by means of a calibration curve as (+) catechin standard (mg/L).

2.2.4. Antioxidant activity

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Potential antioxidant activity has been evaluated using the method developed by Prieto, 2012 [22] with some modifications. DPPH solution 0,2 mM have been prepared pured DPPH powder dissolved in EtOH. This solution must be kept in the fridge wrapped in foil when not in use, to reduce its degradation.

The analysis has been performed in microplate reader using a 96-well microplate in which has been added 100 µL of sample and 100 µL DPPH 0,2 mM. Serial dilutions (1:2) of samples can be done using EtOH. As standard has been used Vitamin C in different dilution. In addition, for each sample and standard, a blank has been prepared by adding only 100 µL EtOH instead of DPPH solution. Moreover, the maximum signal of the radical has been obtained by reacting 100 µL DPPH solution with 100 µL EtOH.

Then the plate has been covered with the lid to minimize evaporation and it has been incubated for 30 minutes. The absorbance (517 nm) has been then read in a microplate reader. The percentage of radical scavenging can be obtained using the following expression (1):

$$\% \text{ DPPH scavenging} = 100 \times [(\text{Abs Sample} + \text{DPPH}) - (\text{Abs Sample Blank})] / [(\text{Abs DPPH}) - (\text{Abs Solvent})] \quad (1)$$

2.2.5. ACE inhibitory activity

Angiotensin I Converting Enzyme (ACE) Activity Assay Kit (Fluorometric) (Merck-Sigma-Aldrich, Milano, Italia) has been used to assess total ACE inhibitory activity of YoAlp® whey-based beverages. Samples were diluted 1:2 in the Assay Buffer and ACE positive control has been used. The assay has been performed in excitation mode at 320 nm and emission mode at 405 nm at 37°C. Fluorescence has been read immediately in kinetic mode in 5 cycles for 5 minutes. Results has been obtained using a standard curve and a sample kinetic curve. The standard curve linear regression slope and the sample kinetic curve linear regression slope were used to transform values of samples from RFU/min to nmol/min (units) using the following formula (2):

$$\text{sample enzymatic activity (nmol/min)} = ((\text{slope sample}) / (\text{slope standard})) * \text{Dil.fact} \quad (2)$$

A percentage of inhibition was then obtained from the following formula (3):

$$\% \text{ inhib.} = ((\text{Fpc-Fsamp}) / (\text{Fpc-Fnc})) * 100 \quad (3)$$

Where Fpc is the fluorescence of positive control; Fsample is the fluorescence of the sample and Fnc is the fluorescence of the negative control.

2.3. Microbiological analysis

3M Petrifilm™ Count Plate specific for different microorganisms (3M Company, St. Paul, MN, USA) have been used to assess total viable cell counts of bacteria, yeasts and mold, *Escherichia coli* and coliforms.

Plates have been incubated for 72 hours at 30° C for total aerobic and yeast and mold count, while an incubation for 24-48 hours at 37°C has been applied for *E. coli*/coliform count.

Furthermore, also probiotics have been evaluated. MRS Agar with Tween 80 and M17 Agar have been used for *Lactobacillus delbrueckii* and *Streptococcus thermophilus* count respectively. Plates have been incubated for 24-48 hours at 40°C in anaerobiotic conditions for *L. delbrueckii* and for the same time at 45°C in aerobic conditions for *S. thermophilus*. The minimum acceptance threshold for probiotic bacteria has been set to 10E6 CFU/mL for *L. delbrueckii* and 10E7 CFU/mL for *S. thermophilus* as reported by Ranhadeera et al., (2017) [31].

2.4. Statistical analysis

All samples have been analyzed in three technical replicates. Results are expressed as the mean (n = 3) ± SD. All the statistical analyses have been performed using Jamovi software (The Jamovi project (2023). Jamovi Version 2.3, Sidney, Australia) and confirmed by JASP software (JASP Team (2023). JASP (Version 0.17.3), Amsterdam, The Netherlands). Data have been tested for normality

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using Shapiro-Wilk normality test and for homogeneity using the Levene's Test for Homogeneity of Variance with default parameters. Since data resulted parametric, the analysis of variance test (ANOVA), followed by Tuckey's post-hoc test ($p < 0.05$) has been performed to evaluate the differences among compared group.

3. Results and discussion

3.1. Chemical analysis

3.1.1. Peptides

Peptides analysis by LC-MS ESI+ on the fraction below 10 kDa of YoAlp® whey and all YoAlp® whey-based beverages detected the presence of 92 different peptides. Thanks to literature research and online database, these peptides have been linked to one or more identification. Each m/z can be related to one or more aminoacidic sequences, so a total of 174 possible aminoacidic sequences has been found. Those peptides were originated from all milk proteins: αs1-casein (αs1-CN), αs2-casein (αs2-CN), k-casein (k-CN), β-lactoglobulin (LGB), α-lactalbumin (LALBA) and from β-casein (β-CN).

A similar number of bioactive peptides have been found among beverages, while differences have been highlighted related to the type of peptides detected. This can be due to different pH detected in the samples at 24 hours (YWR: pH 3,81, YWL: pH 4,10, YWA: pH 3,90) which influence the proteolytic activity of LABs. Nielsen et al. [23] have shown that *S. thermophilus* and *L. lactis* increase proteolytic activity with lower pH values from pH 4,6 to pH 4,3 and pH 3,5. Furthermore, the same author [23] has reported an increase in ACE inhibitory activity.

Furthermore, it has been possible to correlate the identified peptides to a specific potential bioactive effect and to predict the probability of each sequence to exert a supposed bioactive effect.

Among the 174 aminoacidic sequences, 35 have been related to a possible bioactive effect. Most of them are considered ACE (Angiotensin-converting enzyme) inhibitory (about 85,29%), the others are supposed to be antioxidant (5,88%), mineral binding, opioid antagonist, cytomodulator, antimicrobial and DPP (Dypeptil peptidase) inhibitory. Peptide Ranker analysis shows that 62,64% of peptides has a low probability to exert a bioactive effect (probability lower than 50%) and 15,52% has a high probability to exert a bioactive effect (probability higher than 75%).

Moreover, the marker of β-Casein A2 variant, the beta-casomorphin BCM-9 (VYPFPGPIPN), related to possible positive effects on human health, has been detected and it has been highlighted the absence of betacasomorphin BCM-7, marker for the β-Casein A1 variant (BCM7), which is a potential risk factor for health diseases. The most interesting bioactive peptides and the marker of β-Casein A2 variant are shown in Table 1. Some of these peptides are supposed to resist to the gastric digestion according to literature and YQEPVLGPVRGPFPIIV has been reported to has been found in blood plasma [24,25].

Table 1: Most interesting bioactive peptides and potential markers.

[M+H]+ m/z	MW	Charge	Sequence	Position	Protein	Biological activity	Peptide ranker
291	290	1	MAA	24-26	β-LG	ACE inhibitor	0,43
375	374	1	PYP	179-181	β -CN	ACE inhibitor	0,85
439	438	1	NIPP	73-76	β -CN	ACE inhibitor	0,64
499	498	1	YGLF	50-53	α-LA	ACE inhibitor	0,95
551	1100	2	VYPFPGPIPN	59-68	β -CN	A2 marker (BCM9)	0,72
555	1109	2	RFFVAPFPE	22-30	αs1-CN	ACE inhibitor	0,64
575	574	1	HLPLP	125-129	β -CN	ACE inhibitor	0,75
585	584	1	YLLF	102-105	β-LG	ACE inhibitor	0,90
621	1862	3	YQEPVLGPVRGPFPIIV	193-209	β -CN	Gastric digestion resistance	0,77
651	650	1	LPYPY	56-60	β -CN	ACE inhibitor	0,83

3.1.2. Polyphenols

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Total polyphenol content of YoAlp® whey-based beverages has been assessed. The highest amount has been observed in YWA (665,99 ± 38,39 mg/L CE) than YWR (132,55 ± 5,18 mg/L CE) and YWL (98,55 ± 2,25 mg/L CE) (Figure 1).

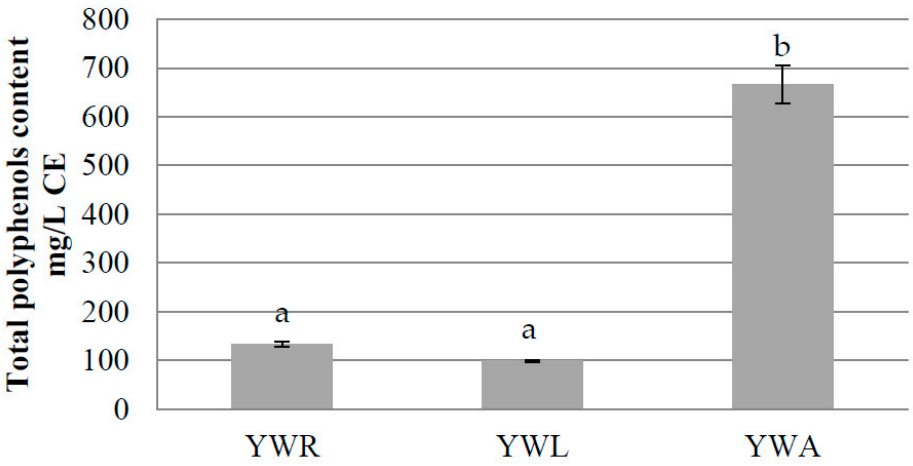

Figure 1. Total polyphenols content (mg/L CE). Different letters indicate statistically significant differences among samples determined by one-way ANOVA followed by post-hoc Tuckey's test ($p < 0.001$).

These results confirmed those obtained in the analysis performed on fruit juices. The richest fruit juice was Renetta/Aronia (2967,59 ± 98,92 mg/L CE), followed by Ravèntse juice (588,17 ± 7,45 mg/L CE) and Renetta/Golden/Raspberry juice (278,44 ± 37,32 mg/L CE).

Statistical significance has been highlighted between group a and group b ($p < 0.001$), while no statistical significance has been found among YWR and YWL.

Moreover, no significative differences on polyphenols content have been detected during the shelf-life period.

3.1.3. Antioxidant activity

Antiradical activity against DPPH was assessed in whey-based beverages and expressed in Vitamin C Antioxidant Capacity Equivalents (CEAC) mg/L. The values detected in all samples ranged from a mean value of 152,43 to 1463,13 mg/L CEAC.

YWA shows the highest mean value of inhibition reaching 1463,13 ± 38,25 mg/L CEAC, more than YWL (186,00 ± 10,17 mg/L CEAC) and YWR (152,43 ± 18,36 mg/L CEAC) (Figure 2). Moreover, also YoAlp® whey without the addition of fruit juices has been analyzed. The results show low values if compared to beverages reaching 20,79 ± 0,69 mg/L CEAC.

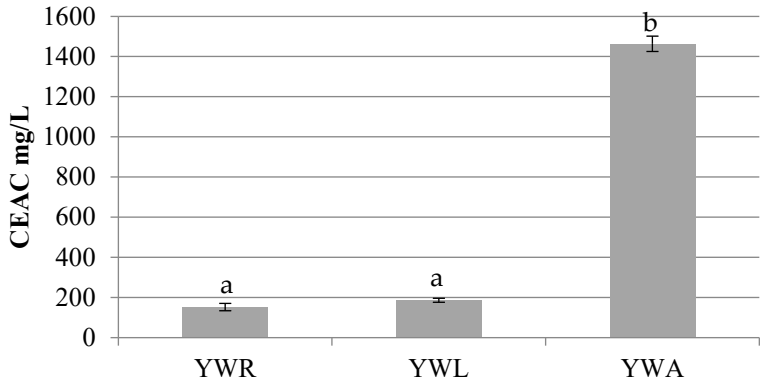

Figure 2. Antiradical power expressed CEAC mg/L. Different letters indicate statistically significant differences among samples determined by one-way ANOVA followed by post-hoc Tuckey's test ($p < 0.001$).

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These results of YoAlp® whey-based beverages suggest a good antioxidant activity if compared with other similar whey-based beverages studied. Jaworska et al., 2014 [32] assessed 76,60 mg/L CEAC on a whey-based Apple beverage (result only available in mg/L CEAC).

Statistical significance has been highlighted between group a and group b ($p < 0.001$), while no statistical significance has been found among YWR and YWL.

Finally, a great correlation between ARP (antiradical power) values and polyphenol content was found indicating that the antiradical power is mainly due to polyphenols present in fruit juices (Figure 3).

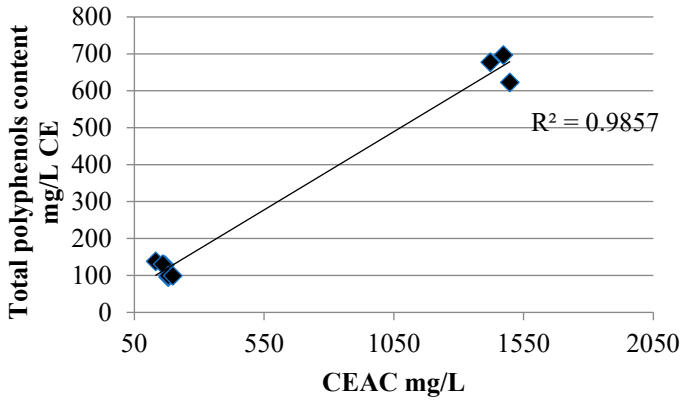

Figure 3. Linear correlation between the antioxidant activity and the concentration of polyphenols.

3.1.4. ACE inhibitory activity

Angiotensin Converting Enzyme (ACE) inhibitory activity was tested in all samples (Figure 4) to verify the biological activity potentially exert by biochemical components, in particular bioactive peptides.

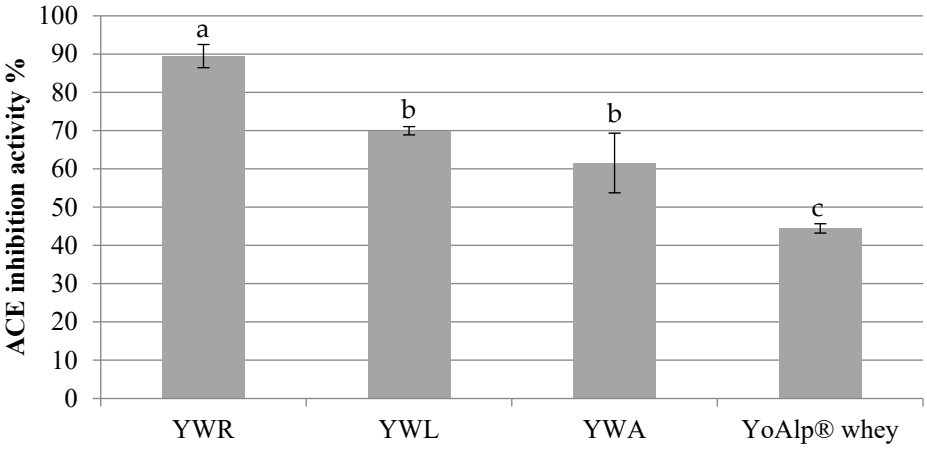

Figure 4. ACE inhibition activity expressed in %. Different letters indicate statistically significant differences among samples determined by one-way ANOVA followed by post-hoc Tuckey's test ($p < 0.005$).

Results showed by YoAlp® whey-based beverages highlighted a percentage of ACE inhibition from 61,55% to 89,50%. The highest value has been reached by YWR (89,50 ± 3,03%), then YWL juice (69,99 ± 1,10%) and YWA (61,55 ± 7,79%). This can be due to a lower pH value of YWR if compared to YWA and YWL (YWR: pH 3,81, YWL: pH 4,10, YWA: pH 3,90), as reported by Nielsen et al. [23]. Moreover, also YoAlp® whey without addition of fruit juices has been analyzed. These results show good values of inhibition reaching 44,46%. So, it is possible to conclude that the ACE inhibitory

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activity is probably due to bioactive peptides present in whey together with phenolic compounds present in fruit juices.

These results were compared with ACE inhibitory activity data of similar studies showing that YoAlp® whey beverages ACE inhibitory activity, in particular whey based Ravèntse beverage has a very high functionality compared with similar products [26–30].

Statistical significance has been highlighted between group a, b and c (a and b $p < 0.002$, a and c $p < 0.001$, b and c $p < 0.005$), while no statistical significance has been found among YWA and YWL.

3.2. Microbiological analyses

Microbiological assessment has been conducted to determine the presence of different categories of microorganisms, and to monitor their variations along shelf life. YoAlp® starters were assessed to verify the vitality and viability of these probiotic bacteria. Spoilers and pathogens were also detected to avoid their presence along shelf life.

Microbial count of lactic acid bacteria has been detected along 4 different shelf-life steps from 24 hours to 21 days (Figure 5). The vitality and viability of these bacteria used as starter in YoAlp® making process must be monitored since they are probiotic organisms that confer possible biological activity to YoAlp® whey-based beverages. The microbial charge has slowly decreased during the shelf-life period at storage temperature (4°C).

The count of *S. thermophilus* remains high even after 7 days (9,00E+06 CFU/ml), in particular for YWA with 1,50E+07 a value that allow to exert a probiotic activity. On the contrary, *L. delbrueckii* was below (6,33E+05 CFU/mL) the recommended therapeutic levels (10E+06 CFU/mL) able to assure the survivability of probiotics along the gastric tract [31].

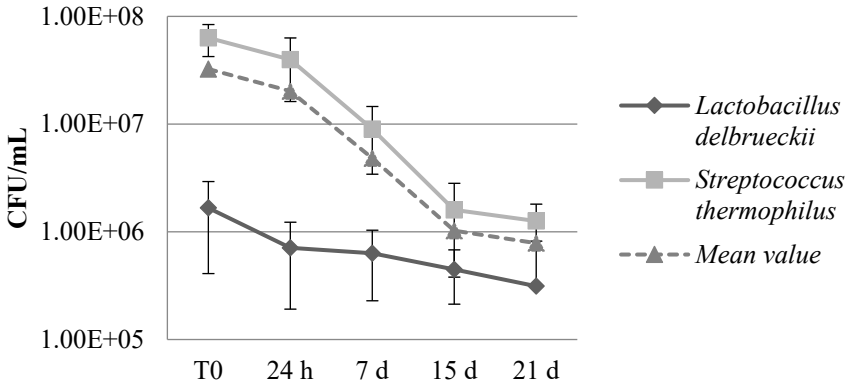

Figure 5. Microbial count of lactic acid bacteria (*L. delbrueckii*, *S. thermophilus* and mean value) along 4 different shelf-life steps from 24 hours to 21 days related to the mean value of the YoAlp® whey based-beverages analyzed.

As far as other microorganisms are concerned, the total microbial count (CMT) was below 10E2 CFU/mL during the entire shelf-life. Coliforms were always under limits and no colonies of *E. Coli* have been detected in any sample.

Considering yeasts, during the first steps, they were under control maintaining always counts below 10E2 CFU/mL. However, after 15 days of shelf-life, they increased, reaching values over 10E3 CFU/mL. Yeasts at these concentrations could lead to unwanted fermentations during the product storage.

Considering these results, shelf-life period of these beverages has been established up to 7 days at storage temperature of 4°C to avoid the presence of yeast and to preserve natural whey probiotic bacteria.

4. Conclusions

The presence of bioactive peptides and phenolic compounds can give to the product a possible antiradical power and ACE inhibitory activity which seem to be higher than other similar dairy-based beverages present on the market or under formulation. Furthermore, the marker of allelic variant A2 of β-Casein and the absence of marker of allelic variant A1 of β-Casein variant suggest possible beneficial health effects leading to a lower incidence of cardiovascular disease and type 1 diabetes, and a reduction in cholesterol and triglycerides. Moreover, these beverages contain viable Lactic acid bacteria (*Streptococcus thermophilus*) which can give beneficial effects on the gastrointestinal tract and spoilage microorganisms stay under the regulation threshold up to 7 days of shelf-life at 4°C. Anyway, other studies should be carried out to increase the vitality and viability of *Lactobacillus delbrueckii* to gain a probiotic effect during the entire shelf-life period. Furthermore, in vivo studies should be performed to evaluate the bioavailability of bioactive peptides and phenolic compounds.

So, YoAlp® whey-based beverages could represent a huge opportunity for Aosta Valley's farms to increase their income and competitiveness reducing environmental impact of dairy industry thanks to reuse of a byproduct.

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Conflicts of Interest: The authors declare no conflict of interest.

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