

FORMULATION, BIOCHEMICAL CHARACTERIZATION AND SHELF LIFE STUDY OF “YOALP” WHEY BASED BEVERAGE CONTAINING FRUIT JUICES PRODUCED IN AOSTA VALLEY

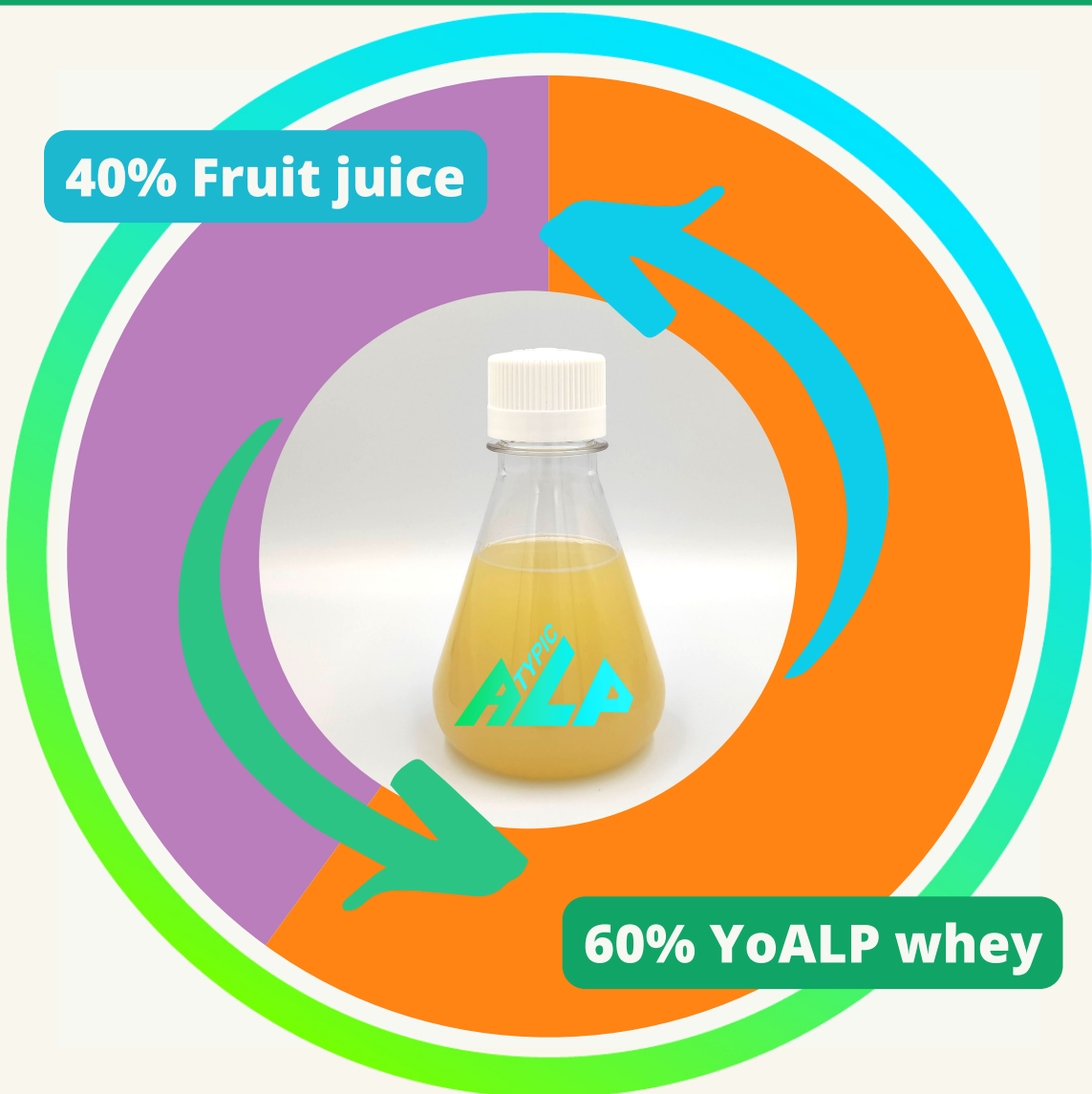


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INTRODUCTION

In order to increase the sustainability of the dairy industry and to respond to a growing demand of functional foods [1], a whey based beverage has been created using “YoALP” whey. “YoALP” is a fermented milk obtained from Aosta Valley cattle breeds milk and local strains of lactic acid bacteria (*Streptococcus thermophilus* and *Lactobacillus delbrueckii*) which have been isolated during years in Aosta Valley mountain dairy cattle farm. From its filtration, by gravity, it is possible to obtain a similar Greek yogurt. During this process acid whey is also produced which is considered a by-product that must be disposed. The disposal of whey is a cost for dairy industries and a loss of important human nutrients [2].

In the formulation of YoALP whey based beverage, in addition to whey, 40% of local fruit juices (apple-Raspberry, apple-Aronia and Ravèntse, an autochthonous apple variety) have been used to improve functionality and flavor. This beverage can be considered functional because it contains components or ingredients able to provide specific health effects other than purely nutritional effect. Health promoting effects depend on nutrients such as polyphenols and bioactive peptides, which potential antioxidant and antihypertensive activity, and on microorganisms with probiotic effects (*Bifidobacterium animalis* spp. *lactis* and *Bifidobacterium breve*).



MICROBIOLOGICAL ANALYSIS

LACTIC ACID BACTERIA

Microbiological analysis (probiotics – MRS agar and M17 agar medium, and Bifidobacteria – HHD culture method) has been conducted to assess the presence of different categories of microorganisms and to monitor their variations along the shelf life. In particular, “YoALP” starters (*Streptococcus thermophilus* and *Lactobacillus delbrueckii*) and Bifidobacteria were monitored up to three weeks so as to verify the presence and vitality of these probiotic bacteria (Fig. 1).

Spoilers and pathogens were assessed (total count – Petrifilm AC, coliforms – Petrifilm EC, yeasts – Petrifilm YM) in order to avoid the presence during a period of twenty-one days.

PROBIOTIC EFFECTS

Streptococcus thermophilus and *Lactobacillus delbrueckii* stay above the recommended therapeutic level and the legal limitation for yogurt of 10E6 CFU/mL up to the seventh day so as to have the ability to tolerate harsh gastric and intestinal conditions and be able to attach to the gut epithelium [3]. Furthermore, these analyses confirmed the safety of the beverages along the entire shelf life (up to 7 days).

RESULTS & DISCUSSION

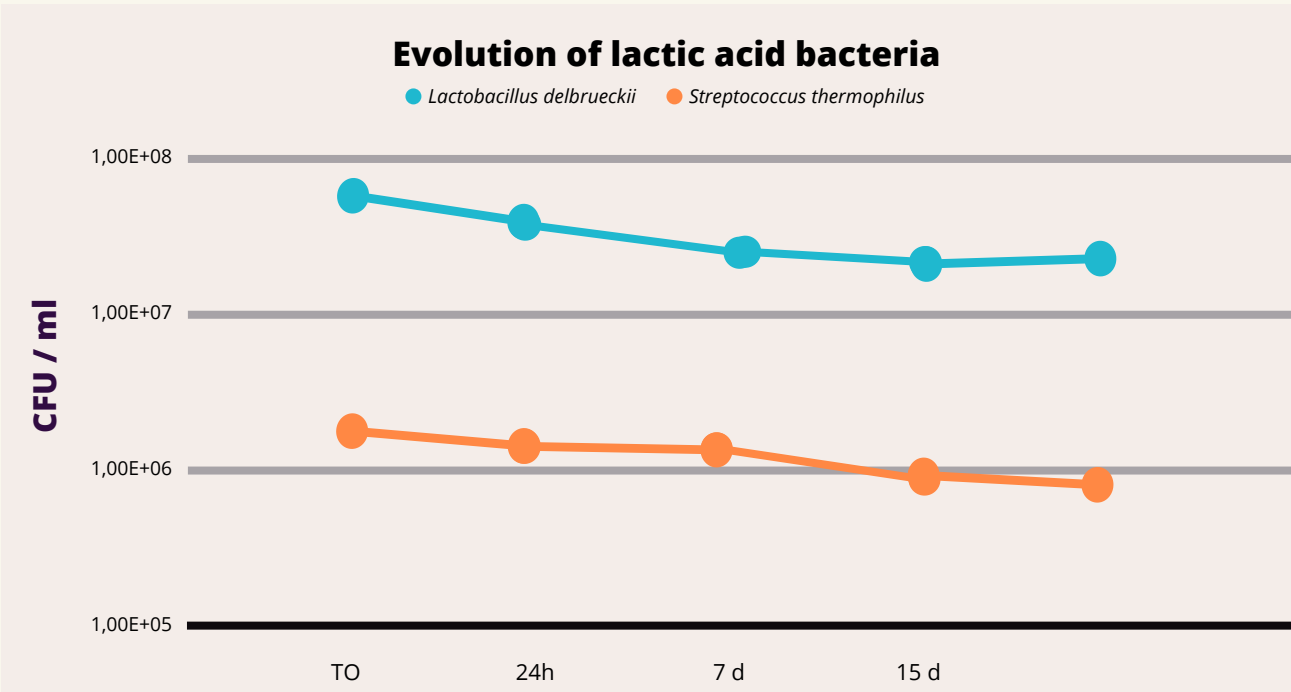


Fig. 1 Evolution of lactic acid bacteria in “YoALP” whey during shelf-life

CHEMICAL ANALYSIS

BIOACTIVES PEPTIDES

Proteomic analysis, conducted by RP-HPLC-ESI (+)/MS using Sforza et al. method with some modifications [4], has highlighted the presence of 118 different peptides from as1-casein (as1-CN), as2-casein (as2-CN), β -casein (β -CN), k-casein (k-CN), β -lactoglobulin (LGB) and α -lactalbumin (LALBA). Among them 30 peptides have been linked to potential bioactive effects such as ACE (Angiotensin-converting enzyme) inhibitory, antioxidant, mineral binding, opioid antagonist and DPP (Dipeptidyl peptidase) inhibitory activity (Tab. 1). ACE inhibitory peptides were the most common bioactive peptides found. This result suggests a possible antihypertensive effect of the “YoALP whey based beverages”. Moreover, it was detected the marker of A2 variant of β -casein (**BCM9**), Tab.1 ID 8, which is related to a lower incidence of cardiovascular disease, type 1 diabetes and a reduction in cholesterol and triglycerides.

ID	RT	[M+H] ⁺ m/z	MW	Charge	Sequence	Origin protein	Biological Activity
1	22,73	585	584	1	YLLF	β -LG (102 - 105)	ACE inhibitor
2	43,7	373	376	1	VYP	β -CN (59 - 61)	ACE inhibitor
3	45,25	439	438	1	NIPP	β -CN (73 - 76)	ACE inhibitor
4	50,13	787	787	1	RELEEL	β -CN (1 - 6)	ACE inhibitor
5	52,14	291	290	1	MAA	β -LG (24 - 26)	ACE inhibitor
6	52,14	375	374	1	PYP	β -CN (179 - 181)	ACE inhibitor
7	57,38	275	274	1	EK	Glu-Lys	ACE inhibitor
8	59,79	864	2589	3	SLVYPPFGPFPINSLPQNPLTQT	β -CN (57-80) *BCM9	ACE inhibitor
9	61,72	665	664	1	VLPQP	β -CN (161-166)	ACE inhibitor

Tab. 1 Identification parameters of peptides detected in YoALP whey beverages and their possible health effects

POLYPHENOLS

Fruit juices contain phenolic compounds known to have a potential antioxidant and free scavenger activity. Normally, a close correlation between the content of phenolic compounds and antioxidant activity should be present in polyphenols rich foods. So, total polyphenolic content and antiradical power (ARP) were determined, respectively by an optimized Folin-Ciocalteu method and a DPPH Antioxidant Capacity Kit (BIOQuochem) which highlighted high values in apple-Aronia whey beverage (Fig. 2).

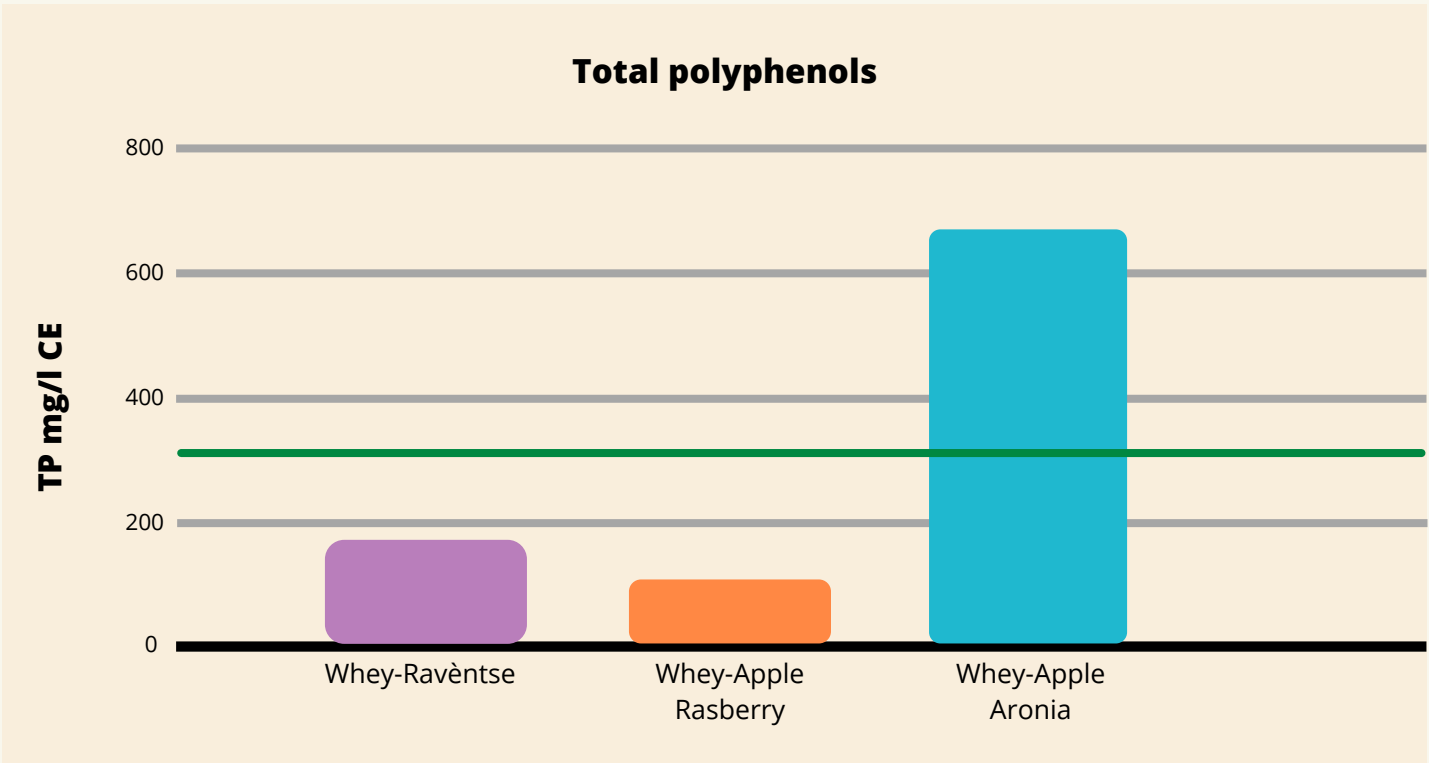


Fig. 2 Total polyphenols content in whey beverages. Mean literature value (—).

ACE INHIBITORY ACTIVITY

In order to verify the Angiotensin Converting Enzyme (ACE) inhibitory activity, potentially exerted by biochemical components, an ACE Activity Assay Kit (Merck-Sigma-Aldrich) was used. Results have shown that this biological activity can be due to ACE inhibitory peptides found in whey and to some polyphenols present in fruit juices. Whey-Ravèntse beverage has reached the maximum inhibition (Fig. 4). In general, all the “YoALP” beverages show higher ACE inhibitory activity than other data present in similar literature studies [5, 6].

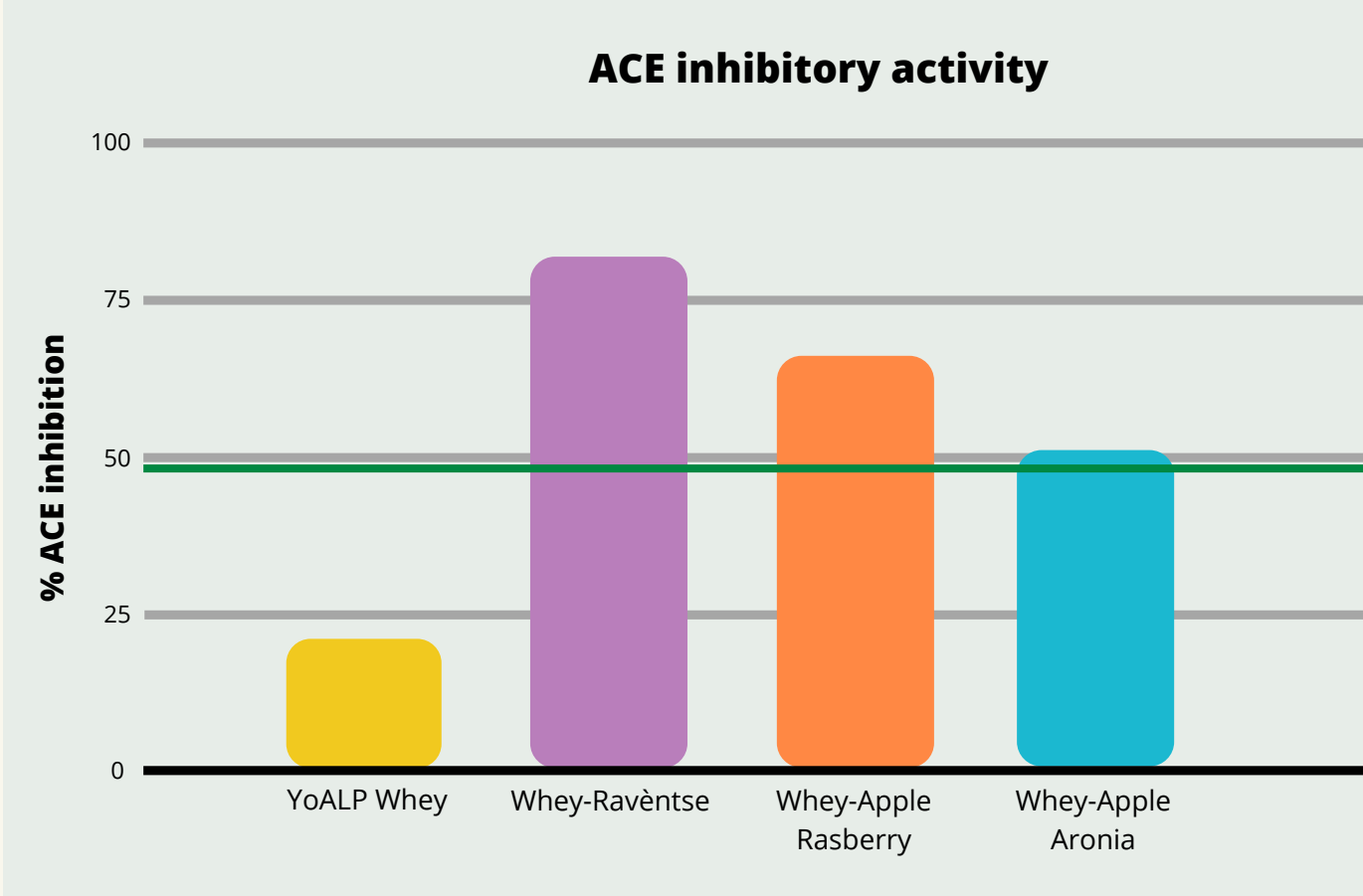


Fig. 4 YoALP whey beverages ACE inhibitory activity. Mean literature value (—).

ANTIRADICAL POWER

High amount of polyphenols was found in all the beverages, in particular, apple-Aronia whey beverage has shown the highest values. As expected, the highest values of ARP have been obtained by apple-Aronia whey beverage. Furthermore, a great correlation between ARP values and polyphenol content was found (Fig. 3) indicating that the antiradical power is mainly due to polyphenols present in fruit juices. Comparing these values with literature, data suggest that YoALP whey based beverages have a good level of potential antioxidant activity [5, 6].

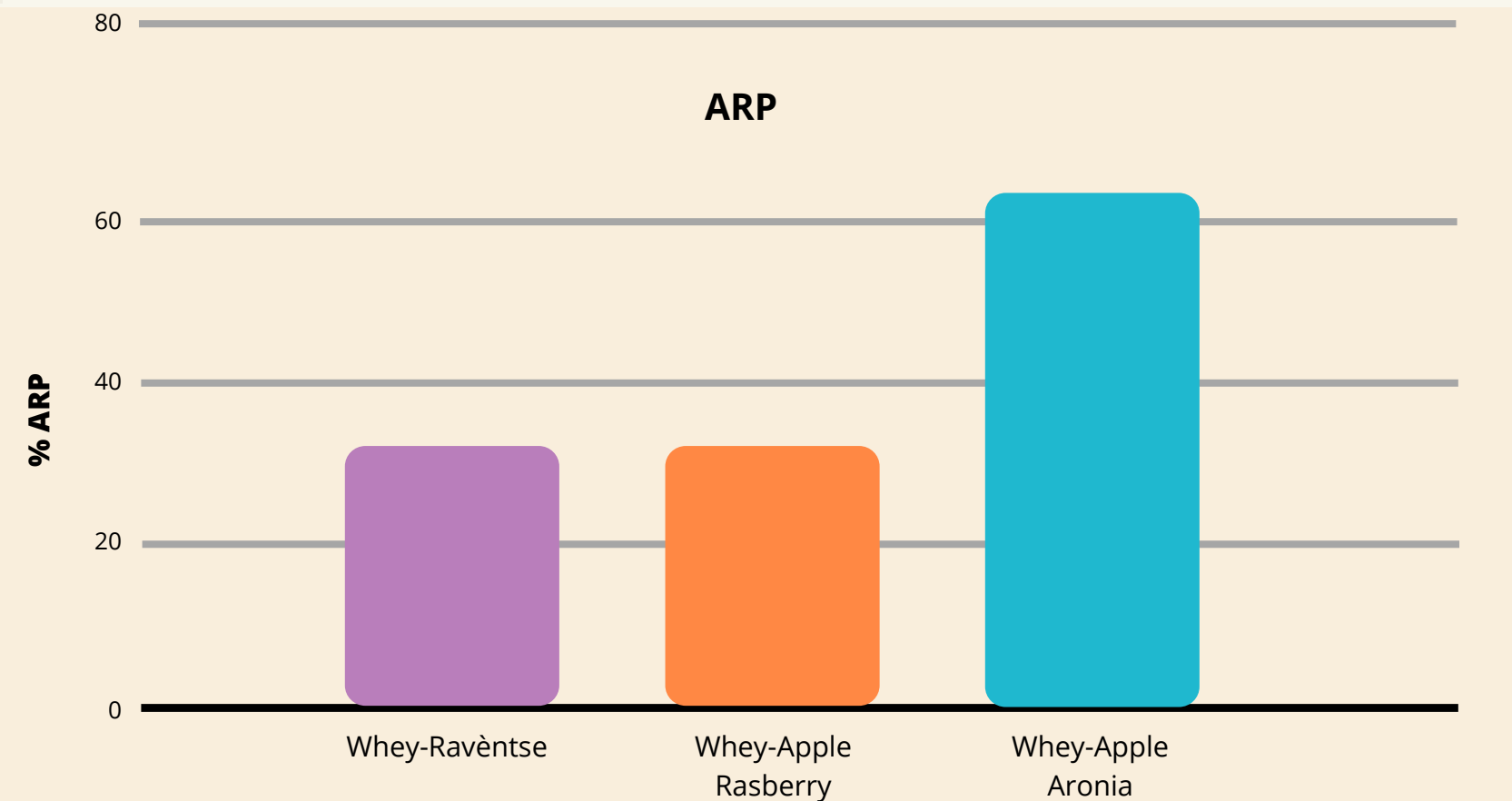
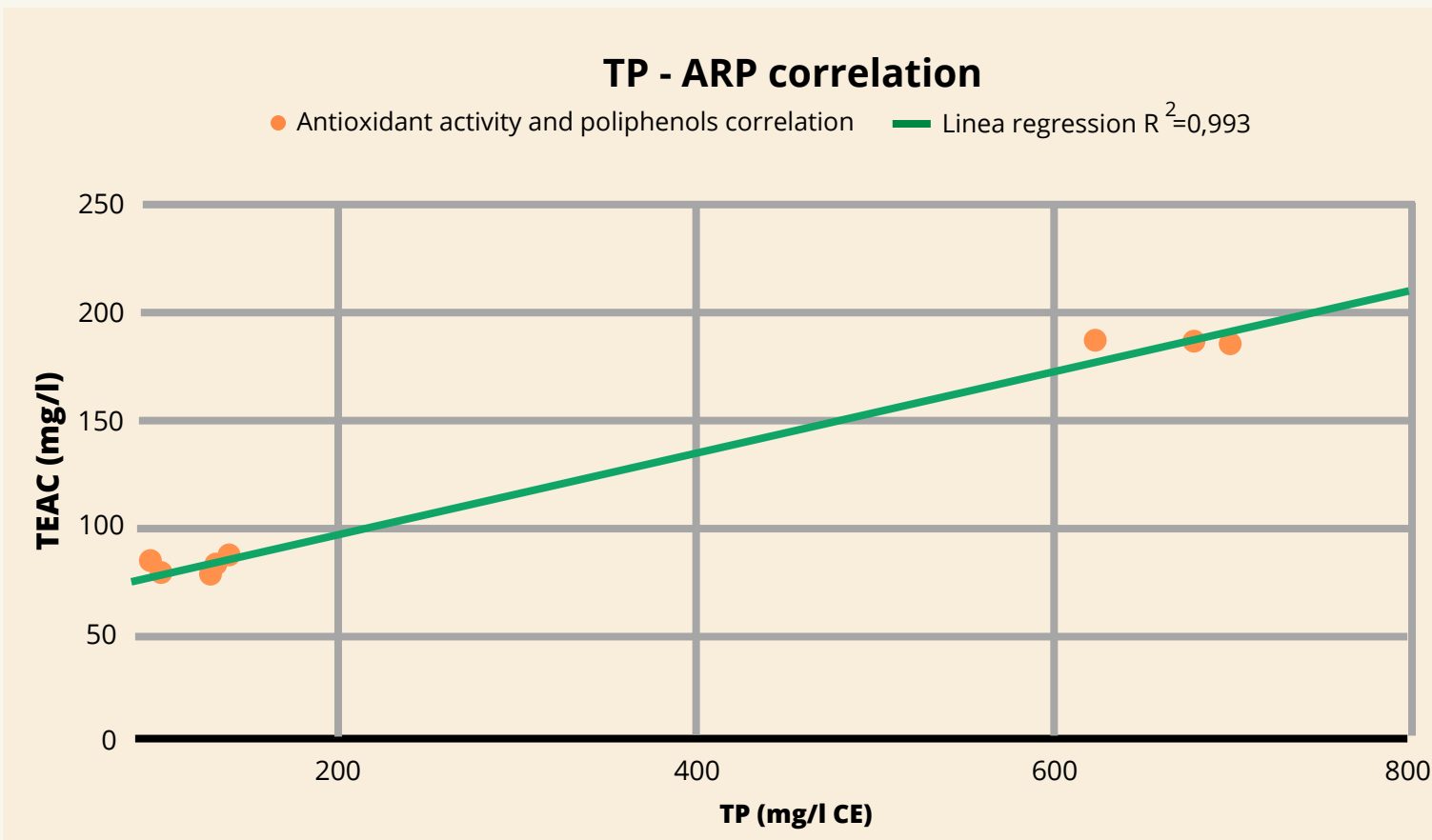


Fig. 3 YoALP whey beverages antiradical power and correlation between total polyphenols and antioxidant activity



CONCLUSIONS

“YoALP” whey based beverages could represent a huge opportunity for the Aosta Valley’s farms to increase their income and competitiveness, creating a circular economy by recovering a by-product like the whey and following the sustainability and healthy trends, which are driving the food sector.

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SCAN ME



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