Identification of Phenolic Compounds in Quinoa Milk Fermented with Lactic Acid Bacteria Strains

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Table of Contents

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Tawanda Mandoga

Abstract

Phenolic compounds, abundant in plants, are of significant interest and are increasingly becoming a subject of intensive research due to their bioactive properties such as antioxidant activity. The objective of this study was to identify phenolic compounds present in formulated quinoa milk fermented by two probiotic bacteria strains; *Lactobacillus pentosus* and *Pediococcus pentosaceous* by HPLC and evaluate their antioxidant capacity. The quinoa milk extract was studied by methods such as Total Phenolic Content using Folin Ciocalteu reagent and scavenging capacity by the ferric acid reducing antioxidant power (FRAP). Other physicochemical parameters including rheology, colour, pH and lactic acid concentration of the milk were also analyzed. From the results of the study, five phenolic compounds were tentatively identified from the formulated quinoa milk. Fermentation of the quinoa milk with lactic acid bacteria increased its phenolic content and antioxidant capacity. Milk fermented with *Lactobacillus pentosus* had highest amount of polyphenols while that fermented with *Pediococcus pentosaceous* exhibited the highest antioxidant capacity. During fermentation bound and conjugated phenolic compounds might have been hydrolyzed by enzymes as well as organic acids (lactic acid) produced by the microorganisms breaking them down into simpler compounds. The fermentation process also increased the viscosity of the milk as well as increasing the whiteness due to the formation of exopolysaccharides.

1. Background

1.1 Health benefits of quinoa

The origin of quinoa (*Chenopodium quinoa* Willd.) is regarded to be in the Andean region of South America. Several quinoa varieties were grown by the pre-Hispanic people for approximately 7000 years (Schoenlechner, 2017) and the crop has had an important impact on these people for centuries due to the nutritional value and health aspects associated with it. Quinoa is very diverse and several different varieties are in existence ranging from yellow, red, white and black genotypes. The crop adapts well to various agro-ecological conditions including semi-arid environments thus it has been recently grown in regions like the USA, England, Sweden, India and some African countries (Schoenlechner, 2017).

Protein quality and quantity of quinoa is relatively superior compared to that of other cereal grains since it offers a gluten-free property and higher digestibility. Protein content of quinoa has a high content of limiting amino acids such as lysine, threonine and methionine when compared to conventional cereals thus makes it a good accompaniment to foods low in these amino acids. Quinoa grain protein content expressed as g/100g edible matter falls between the range 13.1% and 16.7%, these values are higher when compared to those of rice, barley, corn and rye and are close to that of wheat (Vilcacundo and Hernández-Ledesma, 2017). Globulin and albumin are the abundant storage proteins present in quinoa with low concentrations of prolamins making it suitable for gluten free diets. Prolamines such as gliadin from wheat, secalin from rye and hordein from barley; collectively termed glutens, may bring about health issues such as gluten intolerance or gluten sensitivity in some individuals (Graf et al., 2015). Both conditions can trigger an immune mediated enteropathy called coeliac disease, thus quinoa can be considered a safe gluten-free substitute for cereal grains.

Quinoa is rich in polyunsaturated fatty acids such as linoleic and linolenic acid which have been found to help individuals suffering from degenerative diseases such as some cancers, cardiovascular diseases, inflammatory and auto immune diseases (Ludena Urquizo et al., 2017). Quinoa is a good source of vitamins such as riboflavin (B_2), pyridoxine (B_6), ascorbic acid (C), vitamin E, vitamin A and folic acid (B_9) when compared to other grains.

The grain also has high levels of minerals such as magnesium, iron, calcium, zinc and copper when compared to other common grains. Furthermore, calcium, magnesium and potassium are present in quinoa in bioavailable forms hence their contents are considered adequate for a

balanced diet (Vilcacundo and Hernández-Ledesma, 2017). It should be noted that the minerals in quinoa are mainly located in the outer layers of the seed and during the removal of saponins (mechanically or by washing) may reduce some of the mineral content (Schoenlechner, 2017).

1.2 Lactic acid bacteria

Lactic acid bacteria (LAB) is a heterogeneous large group of gram-positive non-spore forming bacteria which is categorised based on a set of morphological, metabolic and physiological characteristics. The main bacteria in this group consists of the genera *Streptococcus*, *Aerococcus, Pediococcus, Leuconostoc* and *Lactobacillus*. LAB are nutritionally fastidious bacteria, thus require a complete nutritional supplementation (Soltan Dallal et al., 2017). The largely require a medium enriched with vitamins, amino acids and lipids for efficient growth. In the food industry, this group of bacteria has been widely used due to certain characteristics they have; LAB has been used as microbial cultures in fermented dairy, beverage, meat and vegetable production.

LAB can be divided into three groups depending on how they utilize sugars; homofermentative, heterofermentative and facultatively heterofermentative bacteria. Homofermentative bacteria such as *Lactococcus*, *Pediococcus* and *Streptococcus* produce lactic acid as the major or only end-product of hexose sugar fermentation. Heterofermentative bacteria can produce equimolar amounts of lactate, CO_2 and ethanol from glucose and facultatively heterofermentative bacteria such as *Lactobacillus* have the ability of fermenting both hexose and pentose sugars (Dujmić, 2017).

Effective fermentation is dependent on the quality of microbial growth and because of this LAB have been widely used, these bacteria can produce several antimicrobial substances that can eliminate a wide range of undesirable organisms in the fermentation process. In some fermented food products LAB increase the vitamin content and nutritional value, phenolic content and digestibility of the food material (Soltan Dallal et al., 2017).

1.3 Fermentation and effect of fermentation on polyphenols

Fermentation is an ancient technology used to improve the shelf-life, organoleptic and nutritional qualities of food. Various biochemical changes occur during fermentation which may lead to altered nutritive content and change in the products properties, such as bioactivity and digestibility (Hur et al., 2014). Recently, fermentation has been applied to the production

and extraction of bioactive compounds in the food, chemical and pharmaceutical industries. In plant based foods and diets fermentation has been applied to increase the content of bioactive phenolic compounds in legumes and other plant sources, thus enhancing their antioxidant activity and overall improved nutritional content.

Microbial enzymes, such as glucosidase, amylase, cellulase, chitinase, produced by fermentation can hydrolyze glucosides, and break down plant cell walls or starch. These enzymes play a role in disintegrating the plant cell wall matrix and consequently facilitating the flavonoids extraction. Since fermentation can improve the antioxidant activity by increasing the release of flavonoids from plant-based foods, it can be considered a useful method for increasing the supply of natural antioxidants.

The fermentation-induced structural breakdown of the plant cell walls may also liberate various bioactive compounds, Katina et al., 2007 and Shyu 2011 as stated by (Hur et al., 2014) have reported that plant parts have an increase in total phenols after fermentation, and that the observed antioxidant activity may be due to the increase in the total phenolic compounds. The phenolic compounds are able to act as reducing agents, hydrogen donors and singlet oxygen quenchers.

Fermentation has a positive influence on the total phenolic content and antioxidant activity of food however, as reported by (Hur et al., 2014) this can also be influenced by various factors, including the microorganism species, pH, temperature, solvent, water content, fermentation time, kind of food and aerobic conditions.

1.4 Polyphenols

Phenolic compounds are secondary plant metabolites that are important determinants in the nutritional and sensory quality of vegetables, fruits and other plants. Together with vitamins phenolic compounds are regarded as important dietary antioxidants. Apart from having an antioxidant effect phenolic compounds contribute to the pigmentation of plants, provide protection against pathogens, predators and external stress such as UV light. Phenolic compounds possess one or more aromatic ring (phenyl) with one or more hydroxyl (OH) groups bound to it (Ioana Ignat, 2011). The presence of the aromatic ring makes them weak acids generating an inductive effect in the hydrogen of the hydroxyl group, thus the aromatic ring plays an important role in the antioxidant properties.

Thousands of phenolic compounds are found in plants and comprise of a variety of structures, these can be phenolic acids and phenolic alcohols (compounds with one phenol ring) or polyphenol structures (having several hydroxyl groups bound to aromatic rings) (Penarrieta, 2009). Phenolic compounds in plants can be generally divided into three groups which are; phenolic acids, flavonoids and tannins.

Phenolic acids can be divided into two classes; hydroxybenzoic acids (derivatives of benzoic acid) such as gallic acid, and hydroxycinnamic acids (derivatives of cinnamic acid) such as coumaric, caffeic and ferulic acid(Ozcan et al., 2014). Hydroxybenzoic acids generally have the structure of C_6-C_1 and may occur is the soluble form conjugated with sugars or organic acids as well as bond to cell fractions. Caffeic acid is the most abundant phenolic acid in many fruits and vegetables.

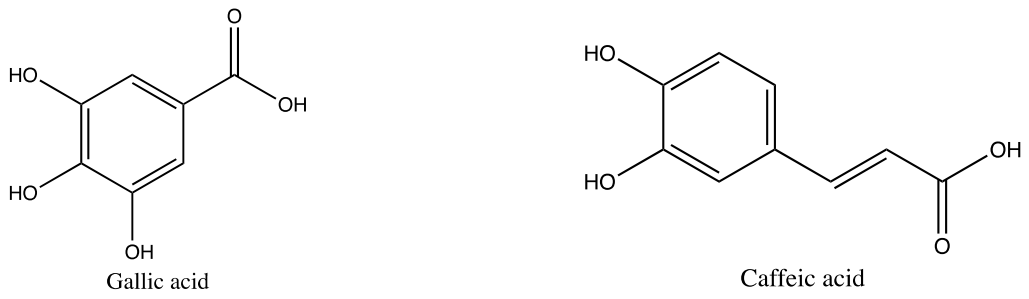


Figure 1. Typical structure of phenolic acids; Hydroxybenzoic acid (gallic acid) and Hydroxycinnamic acid (caffeic acid) (Skowyra, 2014)

Flavonoids are the most abundant polyphenols in our diets and account to over half of the naturally occurring phenolic compounds. These compounds are largely found in fruits and vegetables and give them their colour and flavour. The basic flavonoid structure is the flavan nucleus, containing 15 carbon atoms arranged in three rings (C6-C3-C6) known as the diphenylpropane skeleton, and are usually labelled as A, B and C with variations in the heterocyclic C-ring (Jakesvic, 2004). Flavonoids are themselves divided into six subgroups: flavones, flavonols, flavanols, flavanones, isoflavones, and anthocyanins. The structural variation within each subgroup is partly due to the degree and pattern of hydroxylation, methoxylation, prenylation, or glycosylation (Dai and Mumper, 2010). Some well-studied flavonoids include quercetin, a flavonol abundant in onion, broccoli, and apple; catechin, a flavanol found in tea and several fruits; naringenin, the main flavanone in grapefruit and cyanidin-glycoside, an anthocyanin abundant in berry fruits

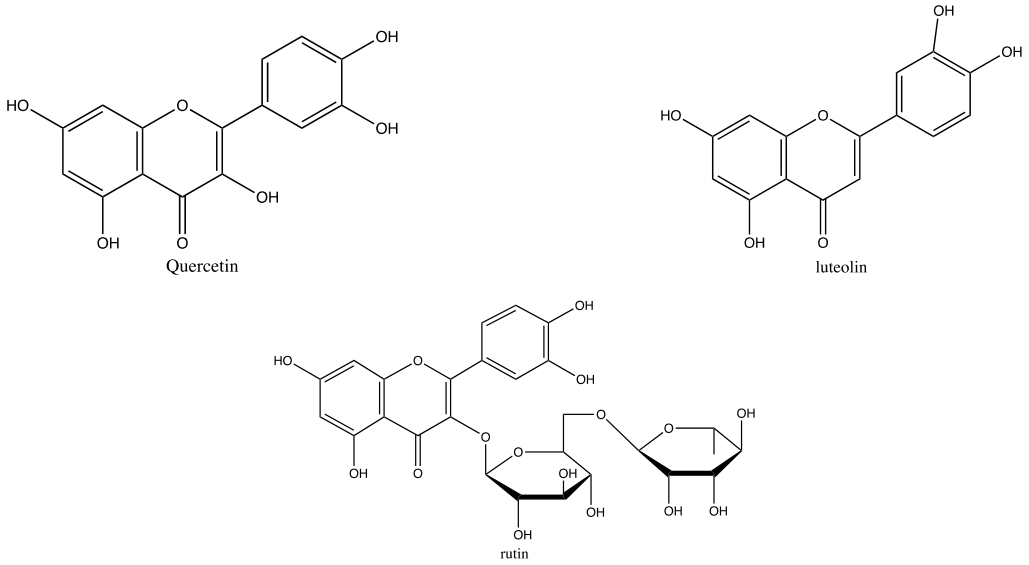


Figure 2. Typical structure of Flavanoids; Flavonol (Quercetin) and Flavone (Luteolin and Rutin)(Skowyra, 2014)

1.5 Antioxidants

Antioxidants are chemical compounds that are present in nature in many plants and food, but can also be synthesised. Antioxidants have the capability to delay, retard or prevent the development of rancidity in food or other flavour deterioration due to lipid oxidation (Yanishlieva et al., 2001). This is carried out by several mechanisms such as binding of metal ions, scavenging of free radicals, absorbing UV radiation and intercepting singlet oxygen. Synthetic antioxidants have been used in the food industry for several decades to maintain the quality of products principally by preventing lipid degradation (Penarrieta, 2009).

Autoxidation of a product may occur in three stages initiation, propagation and termination, (figure 3). The beginning of the oxidation is the initiation stage this is when free radicals are formed from the abstraction of hydrogen in unsaturated fatty acids (RH) due to the influence of UV radiation, heat, metal catalysts and enzymes(Penarrieta, 2009). In propagation, the free radicals react with oxygen to produce peroxide free radicals (ROO$^{\bullet}$) which abstract hydrogen from another unsaturated fatty acid forming hydroperoxide (ROOH) and new free radical (R$^{\bullet}$). The hydroperoxide breaks down to give more free radicals which continue to attack other unsaturated fatty acids with further propagation, until no hydrogen is present or it is interrupted by antioxidants. During termination, the free radicals react with each other to form non-radical products and secondary oxidation products like aldehydes and ketones responsible for rancid off flavours (Indrasena and Barrow, 2010).

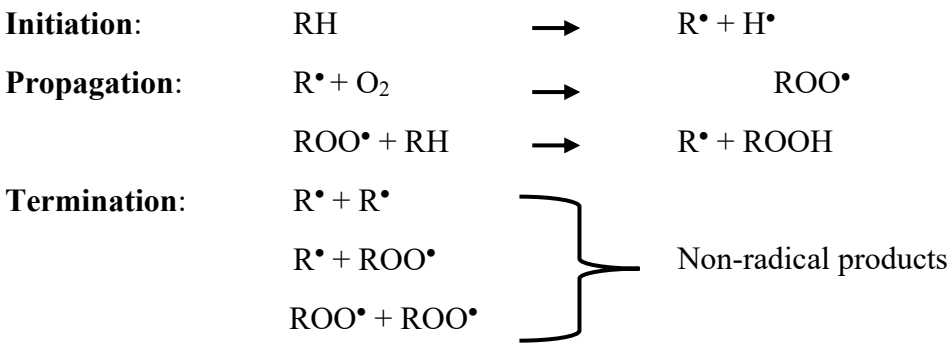


Figure 3. Free radical chain reaction during lipid oxidation process

The theory concerning antioxidants is that they have various properties that can prevent oxidation due to their ability to trap free radicals and retard lipid peroxidation, at either the initiation or propagation stages. Vitamin C, E and phenolic compounds are the main antioxidant compounds found in plant foods. The antioxidant capacity of phenolic compounds is based on their ability to scavenge free radicals and by their binding of metals (Penarrieta, 2009).

2. Aim

The aim of the study is to identify phenolic compounds by HPLC and evaluate their antioxidant capacity from Quinoa milk fermented by two probiotic bacteria; *Lactobacillus pentosus* and *Pediococcus pentosaceus*.

3. Materials and Methods

HPLC was used to identify phenolic compounds present in the fermented quinoa milk. Other parameters like viscosity, colour, pH and lactic acid concentration were also analysed to evaluate physicochemical properties of the fermented quinoa milk. The process and methods will be described in detail in the following section.

3.1 Formulation and fermentation of quinoa milk

The preparation of the quinoa milk samples was carried out in a food-grade lab. The formulation of the fermented quinoa milk was done considering a series of pre-trails to obtain the correct product formulation. Parameters that were considered in the formulation included: washing time, heating temperature, proportion of quinoa and water, mixing speed and time and method of filtration.

The quinoa grains were washed three times under running water, soaked in distilled water in the ratio 1:2 (w/v) for 15 minutes, drained through a sieve and rinsed twice under running water. The drained quinoa grains were roasted until they gave off a distinct nutty smell and a golden colour could be observed. The roasted quinoa was later mixed in a blender with autoclaved Milli-Q water for 4 minutes at speed setting 2 until a homogeneous slurry was obtained. The mixture was filtered through a cheese cloth distributed into 500ml glass bottles and stored at 4°C overnight before being inoculated.

Six bottles containing quinoa milk were separated per bacterium and inoculated with 8.3×10^{8}cfu/mL of *Lactobacillus pentosus*, and with 1.1×10^{9}cfu/mL *Pediococcus pentosaceous*. The quinoa milk was fermented at 30°C during 48 hours in anaerobic conditions. Aliquots of 45 mL of samples was withdrawn at 0 hours, 2 days (48 hours), 14 days (336hours) and 28 days (672 hours) for analysis of the pH, acidity, colour, viscosity, identification of polyphenol compounds and antioxidant capacity.

3.2 pH

Before sampling the pH of the quinoa milk and fermented quinoa milk was measured using a pH meter (Metrohm International, Switzerland).

3.3 Acidity

Lactic acid was used as a measure of acidity since its produced by LAB during the fermentation process. Lactic acid determination was carried out using an Enzytech D-/L- Lactic acid kit obtained from R-Biopharm (Darmstadt, Germany). The samples were analysed following the manufacturer recommendations using a Spectrostar Nano multiplate reader (BMG Labtech, Germany) in triplicates.

3.4 HPLC analysis of polyphenols

The method developed by Gomez-Caravaca *et al*. (2011) was used for the identification of phenolic compounds in the fermented quinoa milk with some modifications. In brief, 2mL of the quinoa milk sample was transferred to a 45 mL eppendorf tube and 13 mL of methanol was added. The samples were then placed on a shaker for 30 minutes and later centrifuged at 4700 rpm for 10 minutes. The supernatant was transferred to a new tube. The residue was again extracted with 15mL of methanol for 24 hours with constant shaking. The samples were centrifuged at 4700 rpm for 10 minutes and the supernatant were collected and mixed with the first extraction. The methanol was evaporated passing nitrogen gas through the sample. After evaporation, the samples were reconstituted adding 2 mL of a mixture of methanol-water (1:1

v/v). The samples were filtered through 0.22 µm syringe filters and stored at 4°C until analysis (Gomez-Caravaca et al., 2011). The sample were analysed before fermentation, after 48 hours' fermentation, and at day 14 and day 28 during storage.

The chromatographic analysis of the quinoa milk sample was carried out using a high-performance liquid chromatography (HPLC 1260, Agilent technologies, Waldbronn Germany), equipped with infinity quaternary LC system, a binary pump, degasser, auto-sampler, a column heater, and a diode array detector (DAD). The separation of the sample was carried out using a Purospher Star Rp-18 endcapped 3µm column. Acidified water (1% acetic acid) as mobile phase A and acetonitrile as mobile phase B were used following the gradient programme in table 1. The total runtime of the program was 30 minutes with a post-runtime of 2 minutes, the flow rate was maintained at 0.8 mL/ min, the sample injection volume used was 5µL, the temperature of the column was used at room temperature.

Table 1. Gradient program for HPLC analysis of quinoa milk samples

Time (min)	Phase A %	Phase B %
0	99.2	0,8
3,5	97,6	2,4
4,5	96	4
6	93,2	6,8
7,5	88,4	11,6
10	87,2	12,8
14	84,6	15,4
19	76	24
28	99,2	0,8

3.5 Total antioxidant capacity (Ferric Reduction Antioxidant Power) Frap assay

The reaction is based on a colour complex formation reduction of yellow ferric TPTZ to blue ferrous TPTZ in the presence of antioxidants. The yellow Fe^{3+}- TPTZ complex is reduced to the blue Fe^{2+}- TPTZ complex by electron donating substances under acidic conditions. Any electron donating substance with half reaction of lower redox potential then Fe^{3+}/ Fe^{2+}- TPTZ will drive the formation of a blue complex.

The FRAP reagent is a mixture of 1,96 g/L sodium acetate buffer acidified with 16 mL acetic acid to pH 3.6, 10 mmol/L TPTZ and 0,54g/100mL Ferric chloride in the ratio 10:1:1 (v/v/v), the reagent was kept in a water bath at 37°C until use. A standard solution of 100 µmol/100mL $FeSO_4 \times 7H_2O$ and was diluted to 100, 200 and 400 µmol/L. To make the calibration curve the 10 µL of the standard solution was placed in the plate reader and allowed to warm up, 260 µL of the reagent solution was then added, and the absorbance was read every 10 seconds for 4

minutes at 595 nm. 10µL of the sample was then added to the plate and left in the reader to warm up for 4 minutes, to which 260 µL of the reagent solution was added to it. The absorbance was then read in a similar manner as that for the standard solution. Samples were read in duplicate and the results were expressed as ferrous sulphate equivalent values (Ring and Chaung, 2015).

3.6 Total phenolic content – Folin Ciocalteu

The total phenolic content of the quinoa milk samples was analysed by carrying out the method described by Burri *et al*., (2017). Gallic acid was used as the standard for result calculations. The Folin Ciocateu reagent oxidises the phenolic compounds to phenolates at alkaline pH in a saturated solution of sodium carbonate resulting in a blue molybdenum-tungsten complex (Penarrieta, 2009).

The assay was carried out by dissolving 75g sodium carbonate in 1 L distilled water and heating over a magnetic stirrer at 50°C for 10 minutes. 100 µL of prepared quinoa milk sample was placed in a cuvette and to that 200 µL of Folin Ciocalteu reagent was added and left to incubate for 4 minutes. Aliquots of 800 µL of sodium carbonate solution was added to the sample mixture and incubated for 1 hour before the absorbance was measured at 765 nm. A Gallic acid standard curve was made (13mg Gallic acid + 5ml ethanol in a 100 mL flask and filled up to the mark with Milli-Q water. 0, 5, 10, 20 and 40µL volumes were used following the same procedure as for the sample with the addition of Milli-Q water to bring the volume of standard sample in the microplate to 100µl. The plate was read at 765nm after incubation time (1 hour). The concentration of Gallic acid per sample was then calculated:

*Concentration of gallic acid (ug/mL per sample) = Abs*dilution*k-value of std. curve* Equation 1

3.7 Rheology

Viscosity

The viscosity of the quinoa milk was characterised using a Kinexus Pro rotational rheometer (Malvern Panalytical, Sweden). The rheometer was equipped with a sensor system of coaxial cylinders (CN 25 0053SS and PC 25 0052AL). A sheer stress ramp was carried out where the shear stress was measured as a function of the shear rate and it ranged from 0.001 to 50 pa, the ramp was carried out within 900 s. The experiment was carried out at 18 °C.

3.8 Colour

The colour of each of the fermented quinoa milk samples was measured using a colorimeter (Konika Minolta, United Kingdom). Measurements were taken by duplicate at room temperature and always in the same location. The parameters used to measure colour are L*, a* and b* and the values represent luminosity (lightness), redness and yellowness respectively (Pathare et al., 2012). L* has a range from 0 to 100, a* takes positive values for reddish colours and negative values for greenish ones and b* takes positive values for yellowish colour and negative for bluish ones. After the measurements, the results were extracted and analysed using Spectra magic software. The colour of the samples was characterised according to the whiteness index (WI) and colour difference values for comparison (ΔE), defined as:

$$WI = 100 - \sqrt{(100 - L*)^2 + a*^2 + b*^2} \qquad \textit{Equation 2}$$

$$\Delta E = [\Delta L^2 + \Delta a^2 + \Delta b^2]^{0.5} \qquad \textit{Equation 3}$$

4. Results

4.1 pH and Lactic acid determination

The changes in pH of the quinoa milk fermented with *Lactobacillus pentosus* and *Pediococcus pentosaceous* are reported in the table 2. The pH values after 48 hours of fermentation were 3,37 for the quinoa milk inoculated with *Lactobacillus pentosus*, and 3,57 for the quinoa milk inoculated with *Pediococcus pentosaceous*.

D-lactic acid and L-lactic acid content of the quinoa milk can be observed in table 2. The highest L- and D- lactic acid content for *Lactobacillus pentosus* was at day 28, followed by day 14 with 7,70g/L and 5,86g/L, respectively. Quinoa milk inoculated with *Pediococcus pentosaceous* had the highest L- and D- lactic acid reading of 6,54g/L and 4,48g/L at day 14.

Table 2. pH and D-, L- lactic acid concentration for samples 1rb1 and 1rd4 from time 0 to 28day storage

	L. pentosus			*P. pentosaceous*		
Day	**pH**	**L- Lactic (g/L)**	**D-Lactic (g/L)**	**pH**	**L- Lactic (g/L)**	**D-Lactic (g/L)**
0	6,59	0,62± 0,57	0,46± 0,34	6,71	0,28± 0,47	1,17± 0,27
2	3,45	7,46± 0,75	5,09± 0,73	3,60	5,52± 2,79	3,02± 1,02
14	3,37	7,58± 1,03	5,86± 1,50	3,57	6,54± 1,07	4,48± 1,27
28	3,43	7,70± 1,09	2,97± 0,65	3,58	5,91± 0,91	3,16± 0,60

The lowest lactic acid readings for both samples was at day 0 which makes sense because fermentation had not yet occurred. There was no significant difference for D-lactic acid values between days 2 and 28 (p= 0,936 and p=0,112 respectively). It was noted that both LAB strains used during the fermentation of the quinoa milk produce higher amounts of L-lactic acid as compared to D-lactic acid.

4.2 Colour

Lightness represented as the L* value of the formulated and fermented quinoa milk samples varied between 77,83 to 82,88 for all the samples. The highest L* value for the quinoa milk fermented by *Lactobacillus pentosus* was 82,13 at day 14 and the whiteness index (WI) was 80,71 at day 28. For the quinoa milk fermented by *Pediococcus pentosaceous* the highest L* value was 82,88 at day 28 and the whiteness index (WI) was 81,46 at day 28.

The total colour difference (ΔE) which represents the overall difference in colour between the freshly prepared quinoa milk and fermented quinoa milk was calculated using equation 3. Based on the classification system highlighted by Cserhalmi *et al*. (2006), ΔE can be categorised according to the following: 0 to 0,5 (not noticeable), 0,5 to 1,5 (slightly noticeable) and >1,5 (noticeable).

Table 3. Colour change based on L* a* b* scale, Colour difference and Whiteness index of samples 1rb1 and 1rd4 between time 0-to-28-day storage.

Probiotic Bacteria	Time (days)	L*	a*	b*	ΔE	WI
Lactobacillus Pentosus	0	77,83 ±0	-1,44 ±0	8,71 ±0	**0,00**	76,13
	2	79,39 ±0,18	-1,21 ±1,3	7,46 ±0,4	**2,01**	78,04
Pediococcus Pentosaeceous	0	79,67 ±0,39	-1,92 ±0,18	10,86 ±0,76	**0,00**	76,87
	2	82,08 ±0,76	-1,21 ±0,08	6,60 ±0,22	**4,95**	80,87

L* Lightness, a* redness, b* yellowness. All values are mean ± standard deviation of 6 replicates

Table 4. Colour change based on L* a* b* scale. Colour difference and whiteness index for fermented quinoa milk at day 2 and day 28.

Probiotic Bacteria	Time (days)	L*	a*	b*	ΔE
Lactobacillus Pentosus	1rb1 2	79,39 ±0,18	-1,21 ±1,3	7,46 ±0,4	**0,00**
	1rb1 14	82,13 ±0,18	-1,43 ±0,04	7,12 ±0,06	**2,77**
	1rb1 28	79,55 ±1,2	-1,27 ±0,14	6,79 ±0,18	**0,69**
Pediococcus Pentosaeceous	1rd4 2	82,08 ±0,76	-1,21 ±0,08	6,60 ±0,22	**0,00**
	1rd4 14	82,59 ±2,4	-1,17 ±0,1	6,65 ±0,8	**0,52**
	1rd4 28	82,88 ±3,05	-1,12 ±0,17	7,03 ±0,63	**0,92**

L* Lightness, a* redness, b* yellowness. All values are mean ± standard deviation of 6 replicates

Noticeable colour differences of >1,5 was observed within the fermented quinoa milk and freshly prepared quinoa milk for both bacterium with values of 2,01 and 4,95 respectively. When comparing the colour difference during storage after fermentation from day 2 to day 28; table 4, there is a noticeable difference (ΔE 2,77) between day 2 and day 14 for sample *L. pentosus*, and a slightly noticeable difference for sample for *P. pentosaceous* at days 14 and 28 (ΔE 0,52 and 0,92 respectively).

4.3 Rheology

The flow behaviour index (n) and the consistency index (K) values of the formulated quinoa milk obtained from fitting the shear stress versus shear rate data to the power law model are presented in table 5. All the quinoa milk sampled exhibited a pseudoplastic behaviour. The apparent viscosity (termed viscosity in the table) was recorded at a shear rate of $50s^{-1}$.

Table 5. Viscosity, flow behaviours index (n) and consistency index (K) of quinoa milk samples.

	Lactobacillus pentosus				*Pediococcus pentosaceous*			
Day	**Viscosity (mPa•s)**	**n**	**K**	**r^2**	**Viscosity (mPa•s)**	**n**	**K**	**r^2**
0	2,67	0,565	2,445	0.99	2,32	0,557	2,467	0.99
2	2,97	0,566	2,435	0.99	2,60	0,562	2,46	0.99
14	2,74	0,567	2,441	0.99	2,44	0,561	2,462	0.99
28	2,62	0,567	2,445	0.99	2,57	0,562	2,461	0.99

4.4 Total phenolic content; Folin

The standard curve obtained from the gallic acid standard at different concentrations is shown in figure 4. The gallic acid equivalents for the different quinoa milk samples were calculated using the equation of the gallic acid standard curve. A lower gallic acid equivalents value depicts lower amounts of phenols within the sample, the results of the different amounts of phenols for the samples per given time are presented in figure 5.

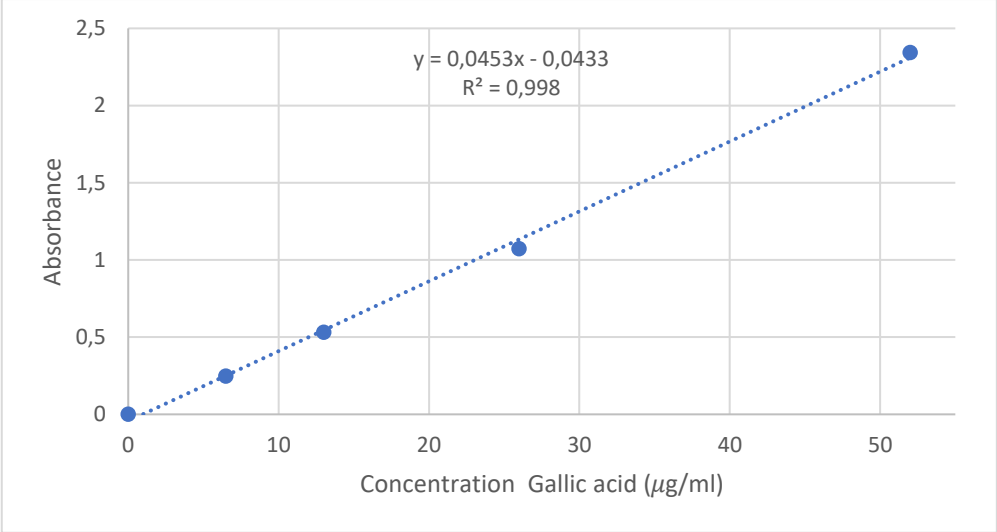


Figure 4. Standard curve for Gallic acid for the determination of total phenolic content

For the quinoa milk inoculated with *L. pentosus*, the smallest amount of phenols is observed at day 0 when the has just been freshly prepared and increases during the fermentation process with values from day 2 to 28 being higher than that of day 0. The highest amount of phenols expressed as gallic acid equivalent was at day 2 (180,61µg/mL) and slightly decreases during the storage period, day 14 and 28 having 176,6µg/mL and 178,07µg/mL, respectively. The total phenols for the quinoa milk inoculated with *P. pentosaceous* was highest during day 0 (207,63µg/mL) and decrease during the fermentation period to their lowest value at day 2 of 163,67µg/mL. However, it can be observed that the phenol content increase slightly from day 2 to day 28 of storage.

Table 6. Absorbance and Total phenolic content as GA equivalent.

	Sample			
	L. pentosus		***P. pentosaceous***	
Day	Absorbance	GA eq (µg/mL)	Absorbance	GA eq (µg/mL)
0	6,02 ±0,61	133,81	9,36 ±3,46	207,63
2	8,14 ±0,86	180,61	7,37 ±0,87	163,67
14	7,96 ±0,64	176,60	7,64 ±1,23	169,63
28	8,02 ±1,01	178,07	8,35 ±1,5	185,34

The initial results of the two quinoa milk samples may vary a bit at day 0 before fermentation due to slight day to day milk sample preparation variations as well as in variations in quinoa boxes purchased.

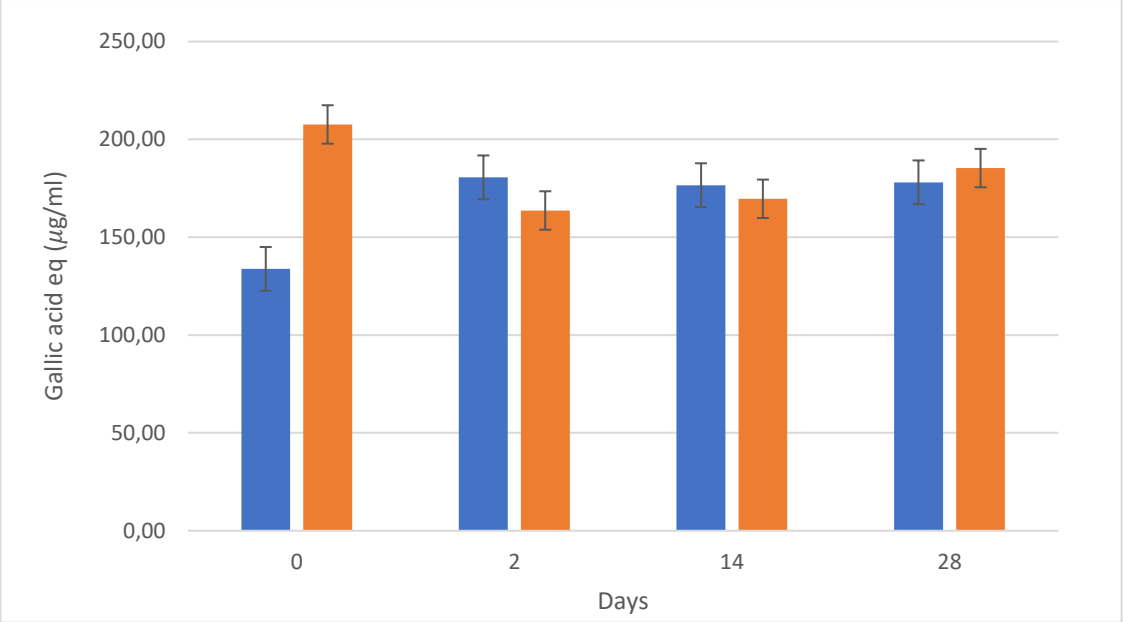


Figure 5. Comparison of total phenols between *L. pensosus* (Blue), and *P. pentosaceous* (Orange) from time 0 to 28 days of storage

4.5 Ferric Reducing Power Potential (FRAP Assay)

The presence of antioxidants in the quinoa milk sample will result in the reduction of the Fe^{3+} to Fe^{2+} by donating an electron. The amount of Fe^{2+} present can be assessed by measuring the formation of a blue colour at 595nm. Figure 6 shows the standard curve obtained from the standard at concentrations 0, 100 200 and 400μmol/L. The ferrous sulphate equivalents for the different quinoa milk samples were calculated using the equation of the ferrous sulphate standard curve.

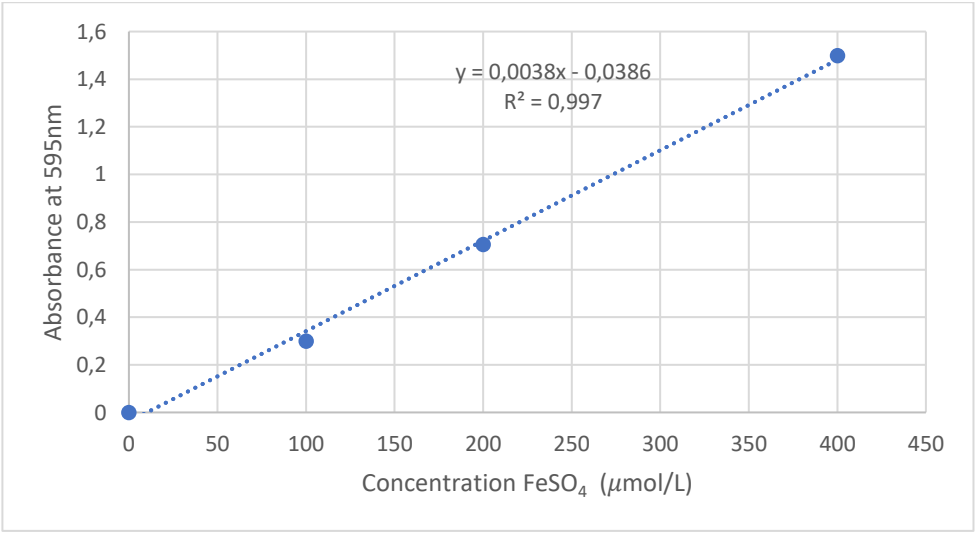


Figure 6. Standard curve for Ferrous sulphate for the determination of antioxidant capacity

The results obtained for the fermented quinoa milk with *L. pentosus* are presented in table 7. It can be observed that there is an increase in the absorbance between day 0 and day 2 (after fermentation). After fermentation and during the storage period till day 28, the absorbance stays constant. Similar behaviour was observed for the quinoa milk fermented with *P. pentosaceous.*

Table 7. Absorbance and Antioxidant capacity as FeSO4 equivalent.

	Sample			
	L. pentosus		***P. pentosaceous***	
Day	Absorbance	$FeSO_4$ eq (μmol/L)	Absorbance	$FeSO_4$ eq (μmol/L)
0	0,18	0,05	0,36	0,10
2	0,23	0,06	0,36	0,10
14	0,20	0,05	0,35	0,09
28	0,20	0,05	0,36	0,10

From previous studies and literature for the FRAP assay an increase in absorbance relatively indicates an increase in the reducing ability of a sample (Aiyegoro and Okoh, 2010), this can also be observed from table 7. *L. pentosus* showed the highest reducing potential at day 2 (0,06). The lowest reducing potential was register at day 14 (0,09) for *P. pentosaceous,* which corresponds to a low absorbance value at that period. Higher reducing potential values (0,10) at day 0, 2 and 28 was register. The reducing power is a meaningful indicator of a compounds antioxidant activity, the amount of phenols in a sample determines how it will be able to reduce radicals in a system thus there a positive correlation between the total phenolic content and FRAP assay when assessing the antioxidant activity of a substance (Molan et al., 2012).

4.6 HPLC

Five distinct peaks were observed for the quinoa milk at time 0 (before fermentation), and 48 hours (after fermentation) according to the chromatograms obtained for *L. pentosus* (Figure 9), and *P. pentosaceous* (Figure 10). Identification of the different peaks was carried out taking in count the retention time, form of the ultraviolet spectrum and the wavelength at the maximum peak and comparing those results with bibliography (Figure 11). The identification indicated the possible presence of two phenolic acids; gallic acid, benzoic acid and three flavonoids; rutin, quercetin and luteolin in the fermented milk samples.

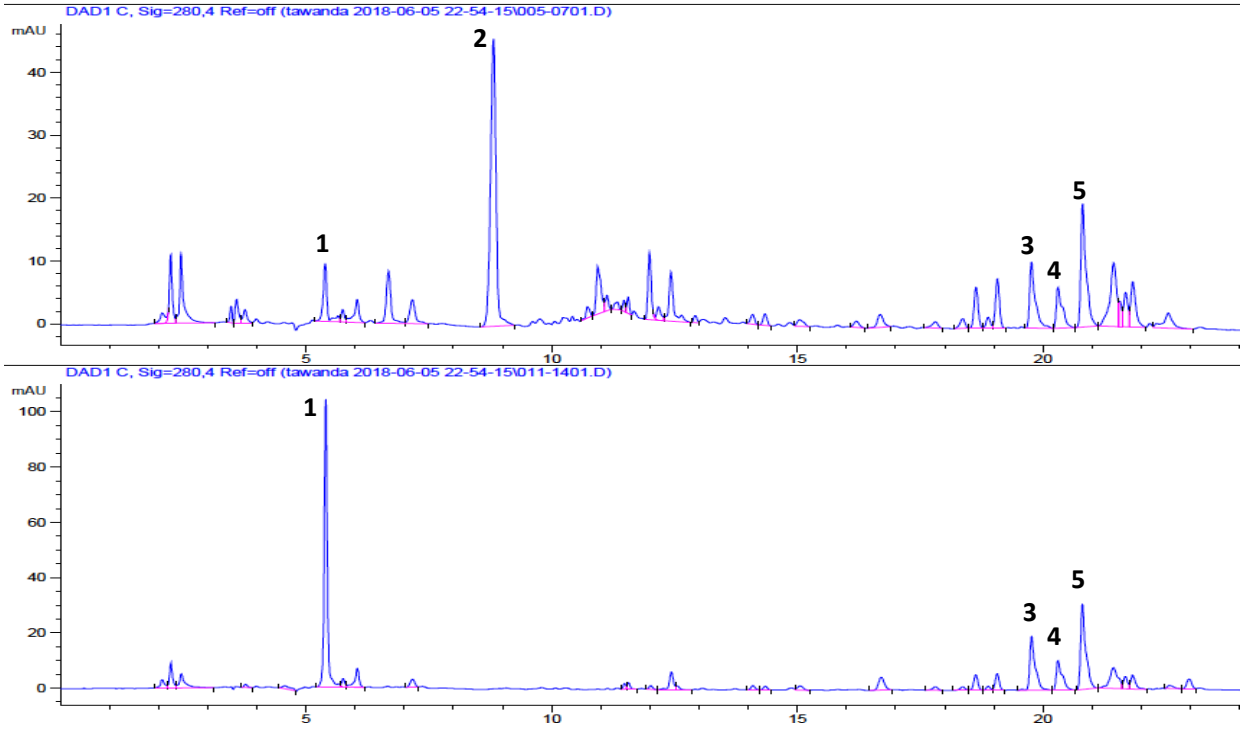


Figure 7. Chromatograms acquired at 280nm of quinoa milk fermented by *L. pentosus* at sampling times before fermentation (above) and after 48 hours of fermentation (below)

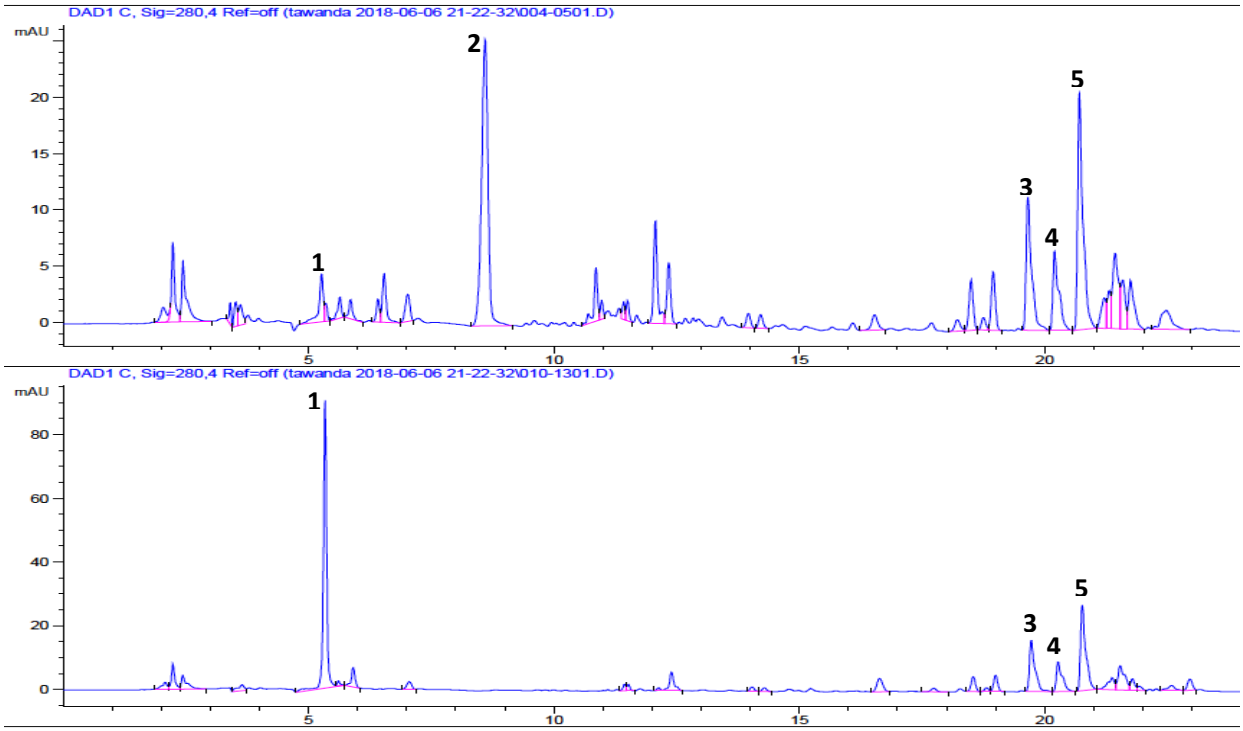
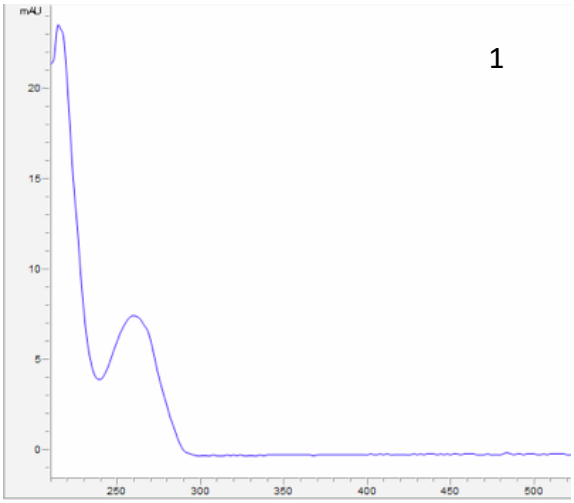
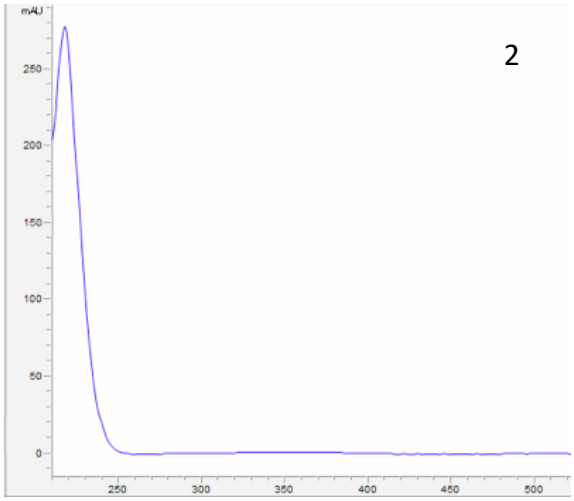


Figure 8. Chromatograms acquired at 280nm of quinoa milk fermented by *P. pentosaceous* before fermentation (above) and after 48 hours fermentation (below)





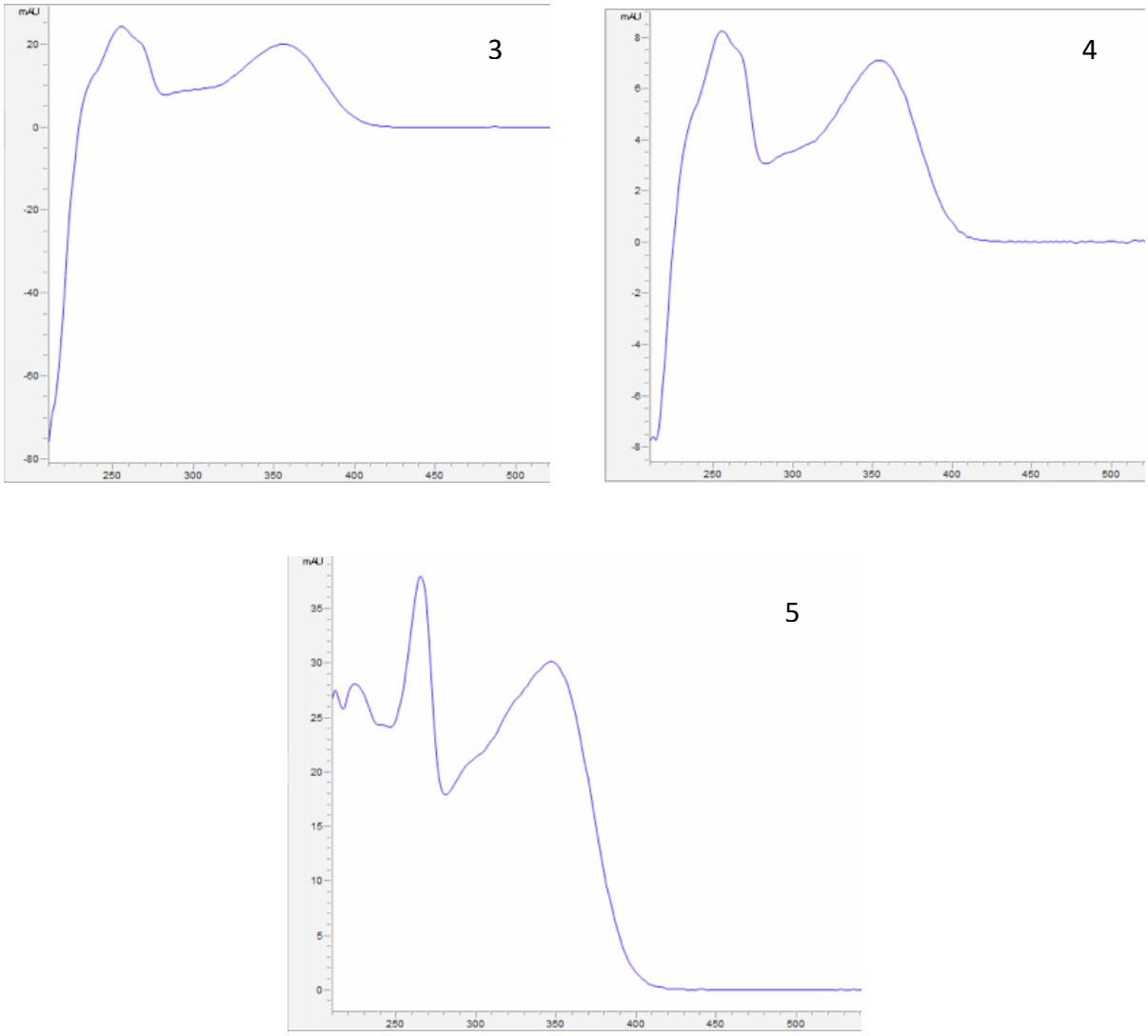


Figure 9. UV-Vis spectra obtained from HPLC-DAD at 280nm in quinoa milk samples, before and after fermentation. Gallic acid (1), Benzoic acid (2), Rutin (3), Quercetin (4), and Luteolin(5).

Table 8 shows the five tentatively identified phenolic compounds from samples inoculated with *L. pentosus*. Table 9 shows five identified phenolic compounds from the quinoa milk fermented with *P. pentosaceous*, as well as the retention times and wavelengths of the UV spectra per compound.

Table 8. Phenolic compounds tentatively identified in fermented quinoa milk inoculated with *L. pentosus*.

Peak #	Retention time (min)	Compound	UV(nm)
1	5,392	Gallic acid	259
2	8,816	Benzoic acid	218
3	19,767	Rutin	257, 356
4	20,309	Quercetin	258, 267, 355
5	20,809	Luteolin	265, 349

Table 9. Phenolic compounds tentatively identified in fermented quinoa milk inoculated with *P. pentosaceous*.

Peak #	Retention time (min)	Compound	UV (nm)
1	5,290	Gallic acid	259
2	8,614	Benzoic acid	220
3	19,680	Rutin	255, 355
4	20,230	Quercetin	256, 266, 353
5	20,730	Luteolin	266, 348

During the fermentation period, there were several changes observed between different peaks of the chromatograms. Chromatograms obtained before and after fermentation with *L. pentosus* were superimposed to clearly show changes (Figure 12). The chromatograms illustrate an increase in the intensity of absorbance of peaks 1, 3, and 5 between time 0 (red) and time 48 (brown) and a decrease in intensity of absorbance for peak 2.

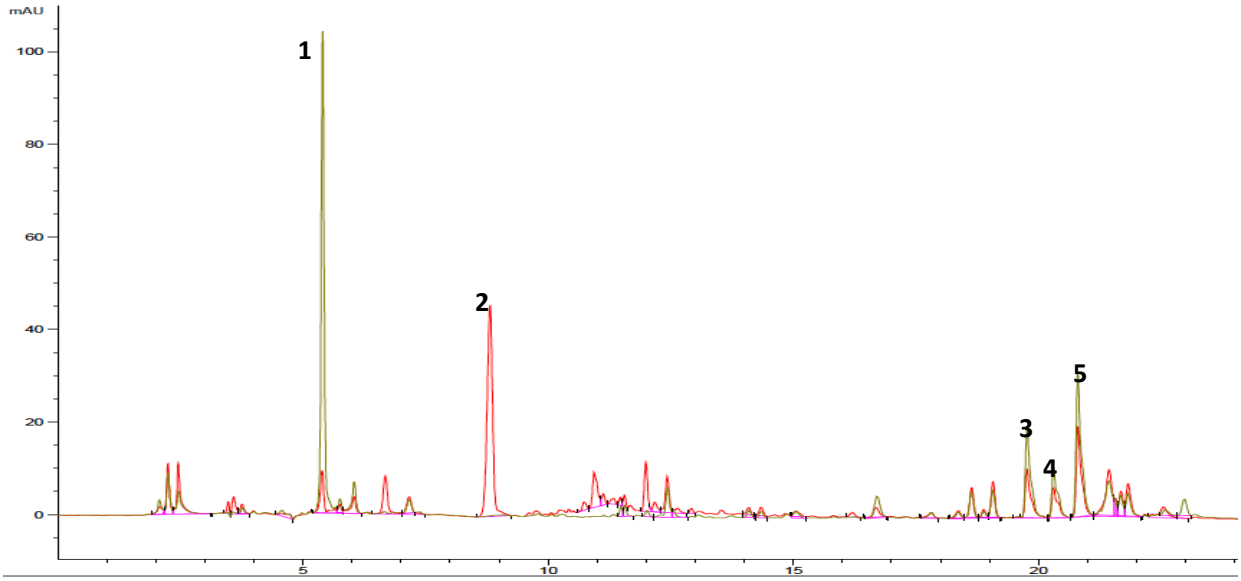


Figure 0. Superimposed chromatograms for *L. pentosus*, before fermentation (red) and after 48 hours fermentation (brown) showing change in peak area

Figure 10 shows the superimposed chromatograms obtained for the quinoa milk fermented by *P. pentosaceous*, at sampling occasions before fermentation and after fermentation to show changes in retention in peak area. There is a decrease in the intensity of absorption for peak 2 for the chromatogram before fermentation (red) and an increase for peaks 1,3,4 and 5 for the chromatogram after the 48-hour fermentation period (brown).

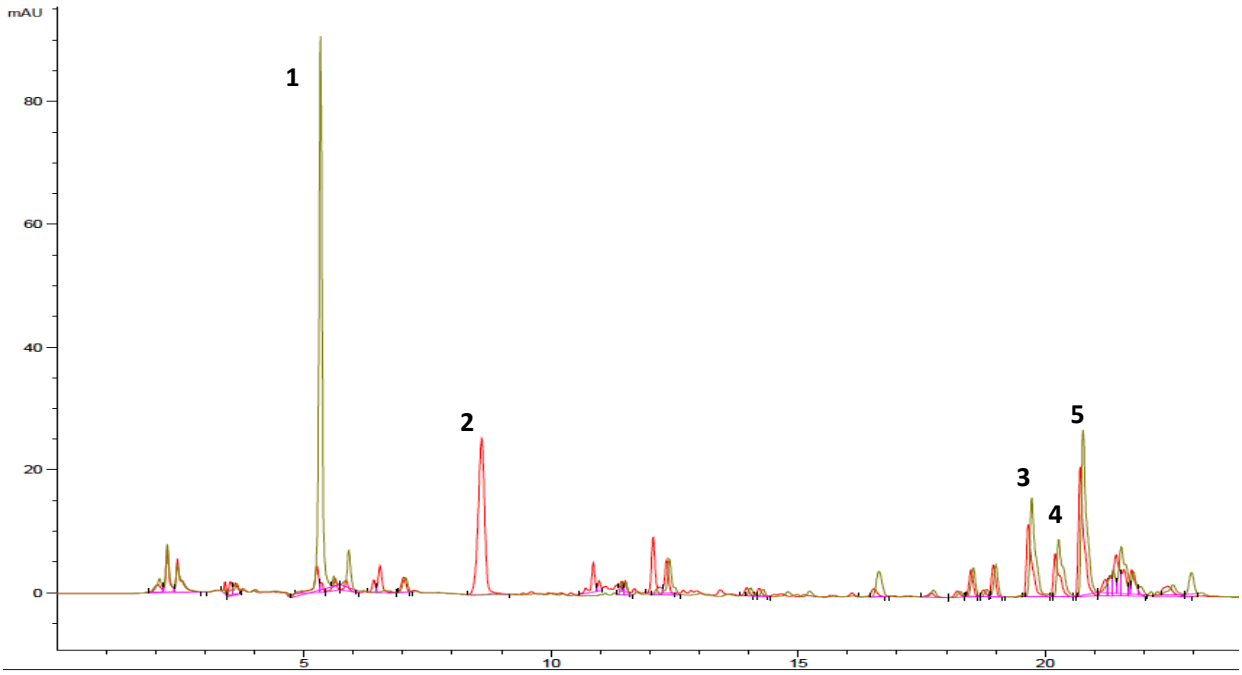


Figure 11. Superimposed chromatograms before fermentation (red) and after 48 hours fermentation (brown) showing change in peak area

5. Discussion

The quinoa grains were cleaned to eliminate possible contaminants carried on during productions to the retailers in Sweden and to the facilities at Lund University. Washing of the quinoa allowed a large amount of saponins to be removed. Some observations were recorded from the cleaning trials. Soaking the quinoa grains for 12 hours resulted in germination of some of the grains as well as soaking up of excessive amounts of water. Moreover, an increased water content in the grains resulted on a poor toasting due to the addition of heat benefit gelatinisation rather than roasting (browning reaction). The partially gelatinised quinoa when blended resulted in a very thick slurry being formed and if left to stand increased in viscosity and made extraction of the milk during filtration difficult. To filter the quinoa milk the cloth was preferred instead of the 250µm metal sieve because the sieve allowed too large particles to pass through which resulted in separation of the milk by sedimentation of the particles, this both affected the colour and rheology of the milk.

The changes in total phenolic content for both samples fermented with *Lactobacillus pentosus* and *Pediococcus pentosaceus* are shown in figure 7. Polyphenols are present in a considerable amount in the quinoa milk after fermentation for the milk inoculated with *L. pentosus* while for *P. pentosaceous* the amount decreases after fermentation. The phenolic content increase

during the fermentation period may be explained by the metabolic activities of *Lactobacillus pentosus* which could have modified the levels of bioactive components such as the different phenolic compounds. During fermentation enzymes, such as B-glycosidase derived from the fermentative microorganism are responsible for the hydrolyzation of complex phenolic compounds to simpler compounds and this in turn increases the quantitative amounts of the total phenolic content. Other enzymes such as protease derived from the microorganisms could have contributed to the modification of milks composition by breaking down proteins. In natural form, phenolic compounds are combined or bound with sugar which reduces their availability to organisms. During fermentation, proteolytic enzymes from the starter organism hydrolyze complexes of phenolics into soluble-free phenols and other simpler and biologically more active ones that are readily absorbed (Adetuyi and Ibrahim, 2014). It has been reported by other researchers that fermentation by lactic acid bacteria or other microorganisms can increase the level of total phenolic content, (Sabokbar and Khodaiyan, 2016). The reduction in the amount of polyphenols after fermentation with *P. pentosaceous* may be due to liquids reduction where polyphenols undergo oxidation facilitated by the enzyme polyphenol oxidase present in the food sample (Cuellar Alvarez et al., 2017), which are responsible for catalyzing the oxidation to high molecular weight condensed polyphenols. This phenomena has been reported in a number of papers (Wollgast and Anklam, 2000).

The antioxidant capacity of a molecule is related to their capacity as electron donors and can reduce the oxidized intermediates of lipid peroxidation processes. This molecules can act as primary or secondary antioxidants (Sabah, 2010). It is generally believed that plants which content high amount of phenolic compounds show good antioxidant activity (Sabah, 2010). A relationship can be observed from the results for the quinoa milk fermented with *Lactobacillus pentosus* where an increase in the total polyphenol content (133,81 GAE μg/ml – 180,61 GAE μg/ml) results in an increase in the antioxidant activity (0,05 $FeSO_4$eq μmol/L- 0,6 $FeSO_4$ eq μmol/L). In contrary, this was not observed for the quinoa milk fermented with *Pediococcus pentosaceus* where a decrease in the total polyphenol content has register. An explanation for this may be that the antioxidant activity of phenolic compounds reportedly varies with the structure and degree of hydroxylation of the aromatic ring (Sabah, 2010). An increase in the number of hydroxyl groups present on the aromatic ring may lead to higher antioxidant activity with the most active possessing 3-6 hydroxyl groups (Pulido et al., 2000), (Sabah, 2010). Although there is a decrease in the phenolic content after fermentation the nature of the

structure of the phenols present in samples fermented with *Pediococcus pentosaceus* may result in increased antioxidant activity; may have more hydroxyl groups present. In comparison samples fermented with *Lactobacillus pentosus* after fermentation may have a large amount of conjugated phenols since the total phenolic content is high but antioxidant activity lower than that of *Pediococcus pentosaceus*. Conjugation of a polyphenol also influences the antioxidant activity, glycosylation of flavonoids reduces their ability to scavenge radicals (Pulido et al., 2000), when the 3-hydroxyl group of quercetin is glycosylated, as in rutin, the result is a significant decrease in antioxidant activity (Sabah, 2010).

The fermentation process for the quinoa milk was carried out for 48 hours and the pH values were 3,45 (*Lactobacillus pentosus*), and 3,60 (*Pediococcus pentosaceus*). One can assume from the reduction in pH and subsequent increase in lactic acid concentration that both LAB reached a considerably high cell density which is a desirable functional characteristic of the fermented milk. *Lactobacillus pentosus* inoculated in the quinoa milk could have had faster adaptation to its environment and a resultant higher production of lactic acid after the 48 hour fermentation period (Lorusso et al., 2018), compared to *Pediococcus pentosaceus* with L-lactic acid concentrations of 7,46g/L and 5,52g/L respectively. During the storage period between day 2 to 28 there is continued production of lactic acid with a relatively constant pH noting the continued activity and survival of *Lactobacillus pentosus* and *Pediococcus pentosaceus*. The increase in lactic acid content and little change in pH may be explained by the buffering ability of the quinoa milk due high amounts of free amino acids, hydrophilic peptides and proteins present which have cationic and anionic groups that can be ionized (Dallagnol et al., 2013).

Color is an important property of foods and plays a key role in food choice; color relates to the quality of products and can affect consumer acceptance. Plant based milk substitutes, generally are extracts of plant material in water which resemble cow's milk in appearance thus consumers expect the product have a pale-yellow color. The whiteness index values for some commercial plant based milks as reported by (Jeske et al., 2017) are 71,35 (quinoa milk), 60,21 (oat milk) and 72,57 (almond milk). The whiteness index values obtained for the quinoa milk before fermentation (76,13 and 76,87) and after the fermentation period (78,04 and 80,87) for *Lactobacillus pentosus* and *Pediococcus pentosaceus* are higher than the reported values. The increase in lightness (L*) and the whiteness index after fermentation may also be a result of the production of exopolysaccharides by the LAB used, exopolysaccharide production increases the opacity of the milk (Bernat et al., 2015). Exopolysaccharides (EPSs) are formed

from monosaccharide residues of sugar and sugar derivatives and are frequently produced by lactic acid bacteria species during fermentation (Sanalibaba and Cakmak, 2016). Exopolysaccharides obtained from LAB are crucial because they help improve the rheology, texture and mouth feel of fermented food products. There was no significant change in the apparent viscosity results for the quinoa milk before and after fermentation one would have expected an increase in the apparent viscosity due to the production of exopolysaccharides. This could have been because of the occasional shaking of the milk that was done during fermentation preventing a bacterial exopolysaccharide network to form within the milk. All quinoa milk samples exhibited a pseudoplastic behavior with a flow index (n) of 0,56, meaning the milk was shear thinning and viscosity decreased with increasing shear rate (Bylund, 2015). Viscosity values were obtained at a shear rate of $50s^{-1}$ since this is the reported average shear rate in the mouth when one is consuming food.

Figures 9 and 10 show the chromatograms for quinoa milk before and after inoculation with *Lactobacillus pentosus* and *Pediococcus pentosaceus*. The chromatograms illustrate the peaks relating to compounds that are eluted from the HPLC between 0 and 25 minutes. A large peak area on the chromatogram correlates to a strong presence of a particular compound while a small peak area correlates to a weak presence of a compound (Brady et al., 2007), the elution times of all the compounds before fermentation and after fermentation are nearly identical in all four chromatograms which indicates a good extraction and separation method. Looking at both superimposed chromatograms; figures 12 and 13 one can observe that there is the decrease in benzoic acid content for the quinoa milk samples after the fermentation process. Increase in the intensity for gallic acid, rutin, querecetin and luteolin on the chromatograms as well as increase in total phenolic content GAE values for the fermented milk further supports the notion that the developed fermented quinoa milk has a higher concentration of phenolic compounds due to enzymatic hydrolysis that would have occurred. Organic acids produced during fermentation cause a in reduction in pH of the quinoa milk and this facilitates acid hydrolysis of some of the bound phenolic compounds freeing them into the milk media thus increasing its phenolic content (Tang et al., 2016). Additionally, other research works (Gomez-Caravaca et al., 2011, Dallagnol et al., 2013, Tang et al., 2016) also report an increase in the amounts of phenolic compounds after fermentation of quinoa with LAB. Samples fermented with *Lactobacillus pentosus* have an overall higher intensity of absorbance values compared to those fermented with *Pediococcus pentosaceus* suggesting that fermenting quinoa milk with *Lactobacillus pentosus* produces more phenolic compounds but as observed from the ferric

acid reducing assay results the higher amount of phenolic compounds produced have no significant influence on the antioxidant activity.

6. Conclusion

Five phenolic compounds were tentatively identified from the formulated quinoa milk. Fermentation of the quinoa milk with lactic acid bacteria increased its phenolic content and antioxidant capacity. Milk fermented with *Lactobacillus pentosus* had highest amount of polyphenols while that fermented with *Pediococcus pentosaceus* exhibited the highest antioxidant capacity. During fermentation bound and conjugated phenolic compounds might have been hydrolysed by enzymes as well as organic acids (lactic acid) produced by the microorganisms breaking them down into simpler compounds. The fermentation process also increased the viscosity of the milk as well as increasing the whiteness due to exopolysaccharide.

References

ADETUYI, F. O. & IBRAHIM, T. A. 2014. Effect of Fermentation Time on the Phenolic, Flavonoid and Vitamin C Contents and Antioxidant Activities of Okra (Abelmoschus esculentus) Seeds. *Nigerian Food Journal,* 32**,** 128-137.

AIYEGORO, O. A. & OKOH, A. I. 2010. Preliminary phytochemical screening and in vitro antioxidant activities of the aqueous extract of Helichrysum longifolium DC. *BMC Complement Altern Med,* 10**,** 21.

BERNAT, N., CHAFER, M., CHIRALT, A. & GONZALEZ-MARTINEZ, C. 2015. Development of a non-dairy probiotic fermented product based on almond milk and inulin. *Food Sci Technol Int,* 21**,** 440-53.

BRADY, K., HO, C.-T., ROSEN, R. T., SANG, S. & KARWE, M. V. 2007. Effects of processing on the nutraceutical profile of quinoa. *Food Chemistry,* 100**,** 1209-1216.

BURRI, S. C. M., EKHOLM, A., HAKANSSON, A., TORNBERG, E. & RUMPUNEN, K. 2017. Antioxidant capacity and major phenol compounds of horticultural plant materials not usually used. *J Funct Foods,* 38**,** 119-127.

BYLUND, G. 2015. *Dairy processing handbook*, Lund : Tetra Pak Processing Systems AB, 2015 (Malmö : Holmbergs)

3. [rev.] ed. / [based on the original textbook by Gösta Bylund ... and updated by a panel of experts from DeLaval ... and Tetra Pak].

CSERHALMI, Z., SASS-KISS, Á., TÓTH-MARKUS, M. & LECHNER, N. 2006. Study of pulsed electric field treated citrus juices. *Innovative Food Science & Emerging Technologies,* 7**,** 49-54.

CUELLAR ALVAREZ, L., CUELLAR ALVAREZ, N., GALEANO GARCIA, P. & SUÁREZ SALAZAR, J. C. 2017. Effect of fermentation time on phenolic content and antioxidant potential in Cupuassu (Theobroma grandiflorum (Willd. ex Spreng.) K.Schum.) beans. *Acta Agronómica,* 66**,** 473-479.

DAI, J. & MUMPER, R. J. 2010. Plant Phenolics: Extraction, Analysis and Their Antioxidant and Anticancer Properties. *Molecules,* 15**,** 7313-7352.

DALLAGNOL, A. M., PESCUMA, M., DE VALDEZ, G. F. & ROLLAN, G. 2013. Fermentation of quinoa and wheat slurries by Lactobacillus plantarum CRL 778: proteolytic activity. *Appl Microbiol Biotechnol,* 97**,** 3129-40.

DUJMIĆ, E. 2017. *POTENTIAL OF SOYA SUBSTRATES FOR LACTIC ACID FERMENTATION WITH ADDITION OF Lactobacillus plantarum.* University of Zagreb.

GOMEZ-CARAVACA, A. M., SEGURA-CARRETERO, A., FERNANDEZ-GUTIERREZ, A. & CABONI, M. F. 2011. Simultaneous determination of phenolic compounds and saponins in quinoa (Chenopodium quinoa Willd) by a liquid chromatography-diode array detection-electrospray ionization-time-of-flight mass spectrometry methodology. *J Agric Food Chem,* 59**,** 10815-25.

GRAF, B. L., ROJAS-SILVA, P., ROJO, L. E., DELATORRE-HERRERA, J., BALDEON, M. E. & RASKIN, I. 2015. Innovations in Health Value and Functional Food Development of Quinoa (Chenopodium quinoa Willd.). *Compr Rev Food Sci Food Saf,* 14**,** 431-445.

HUR, S. J., LEE, S. Y., KIM, Y. C., CHOI, I. & KIM, G. B. 2014. Effect of fermentation on the antioxidant activity in plant-based foods. *Food Chem,* 160**,** 346-56.

INDRASENA, W. M. & BARROW, C. J. 2010. *Oxidation and Stability of Food-Grade Fish Oil: Role of Antioxidants*, Wiley-Blackwell.

IOANA IGNAT, I. V., VALENTIN I. POPA 2011. A critical review of methods for characterisation of polyphenolic compounds in fruits and vegetables. *Food Chemistry,* 126**,** 1821-1835.

JAKESVIC, M. 2004. *Effects of Lactobacillus plantarum 299v on phenolics in rose hip.* Masters degree, Lund University.

JESKE, S., ZANNINI, E. & ARENDT, E. K. 2017. Evaluation of Physicochemical and Glycaemic Properties of Commercial Plant-Based Milk Substitutes. *Plant Foods Hum Nutr,* 72**,** 26-33.

LORUSSO, A., CODA, R., MONTEMURRO, M. & RIZZELLO, C. G. 2018. Use of Selected Lactic Acid Bacteria and Quinoa Flour for Manufacturing Novel Yogurt-Like Beverages. *Foods,* 7.

LUDENA URQUIZO, F. E., GARCIA TORRES, S. M., TOLONEN, T., JAAKKOLA, M., PENA-NIEBUHR, M. G., VON WRIGHT, A., REPO-CARRASCO-VALENCIA, R., KORHONEN, H. & PLUMED-FERRER, C. 2017. Development of a fermented quinoa-based beverage. *Food Sci Nutr,* 5**,** 602-608.

MOLAN, A., FARAJ, A. & MAHDY, A. 2012. Antioxidant activity and phenolic content of some medicinal plants traditionally used in northern Iraq. *Phytopharmacology* 2**,** 224-233.

MORGENSTERN, A., RUMPUNEN, K., SCHEEWE, P. & EKHOLM, A. 2014. Changes in content of major phenolic compounds during leaf development of sea buckthorn (Hippophaë rhamnoides L.). *Agricultural and Food Science,* 23**,** 207-219.

OZCAN, T., AKPINAR-BAYIZIT, A., YILMAZ-ERSAN, L. & DELIKANLI, B. 2014. Phenolics in Human Health. *International Journal of Chemical Engineering and Applications,* 5**,** 393-396.

PATHARE, P. B., OPARA, U. L. & AL-SAID, F. A.-J. 2012. Colour Measurement and Analysis in Fresh and Processed Foods: A Review. *Food and Bioprocess Technology,* 6**,** 36-60.

PENARRIETA, M. 2009. *Antioxidants in Bolivian Plant Foods. Antioxidant Capacity, Flavonoids and other Phenolic Compounds.* Doctoral Thesis, Department of Chemistry, Lund University.

PULIDO, R., BRAVO, L. & SAURA-CALIXTO, F. 2000. Antioxidant Activity of Dietary Polyphenols As Determined by a Modified Ferric Reducing/Antioxidant Power Assay. *Journal of Agricultural and Food Chemistry,* 48**,** 3396-3402.

RING, J. & CHAUNG, M. 2015. The FRAP assay: Allowing students to assess the anti-oxidizing ability of green tea and more. *Journal of Student Science and Technology,* 8.

SABAH, B. 2010. *Antioxidative properties of Tamus communis L., Carthamus caeruleus L. and Ajuga iva L. extracts.* University Ferhat Abbas Setif

SABOKBAR, N. & KHODAIYAN, F. 2016. Total phenolic content and antioxidant activities of pomegranate juice and whey based novel beverage fermented by kefir grains. *J Food Sci Technol,* 53**,** 739-47.

SANALIBABA, P. & CAKMAK, G. A. 2016. Exopolysaccharides Production by Lactic Acid Bacteria. *Applied Microbiology: open access,* 2.

SCHOENLECHNER, R. 2017. Quinoa: Its Unique Nutritional and Health-Promoting Attributes. *In:* AWIKA, J. M. (ed.) *Gluten-Free Ancient Grains.* Woodhead Publishing.

SKOWYRA, M. 2014. *Antioxidant properties of extracts from selected plant materials (Caesalpinia spinosa, Perilla frutescens, Artemisia annua and Viola wittrockiana) in vitro and in model food systems.* PhD, Universitat Politècnica de Catalunya.

SOLTAN DALLAL, M. M., ZAMANIAHARI, S., DAVOODABADI, A., HOSSEINI, M. & RAJABI, Z. 2017. Identification and characterization of probiotic lactic acid bacteria isolated from traditional persian pickled vegetables. *GMS Hyg Infect Control,* 12**,** Doc15.

TANG, Y., ZHANG, B., LI, X., CHEN, P. X., ZHANG, H., LIU, R. & TSAO, R. 2016. Bound Phenolics of Quinoa Seeds Released by Acid, Alkaline, and Enzymatic Treatments and Their

Antioxidant and alpha-Glucosidase and Pancreatic Lipase Inhibitory Effects. *J Agric Food Chem,* 64**,** 1712-9.

VILCACUNDO, R. & HERNÁNDEZ-LEDESMA, B. 2017. Nutritional and biological value of quinoa (Chenopodium quinoa Willd.). *Current Opinion in Food Science,* 14**,** 1-6.

WOLLGAST, J. & ANKLAM, E. 2000. Review on polyphenols in Theobroma cacao: changes in composition during the manufacture of chocolate and methodology for identification and quantification. *Food Research International,* 33**,** 423-447.

YANISHLIEVA, N., GORDON, M. & POKORNÝ, J. 2001. *Antioxidants in Food : Practical Applications,* Boca Raton, Woodhead Publishing.

Appendix Chromatograms

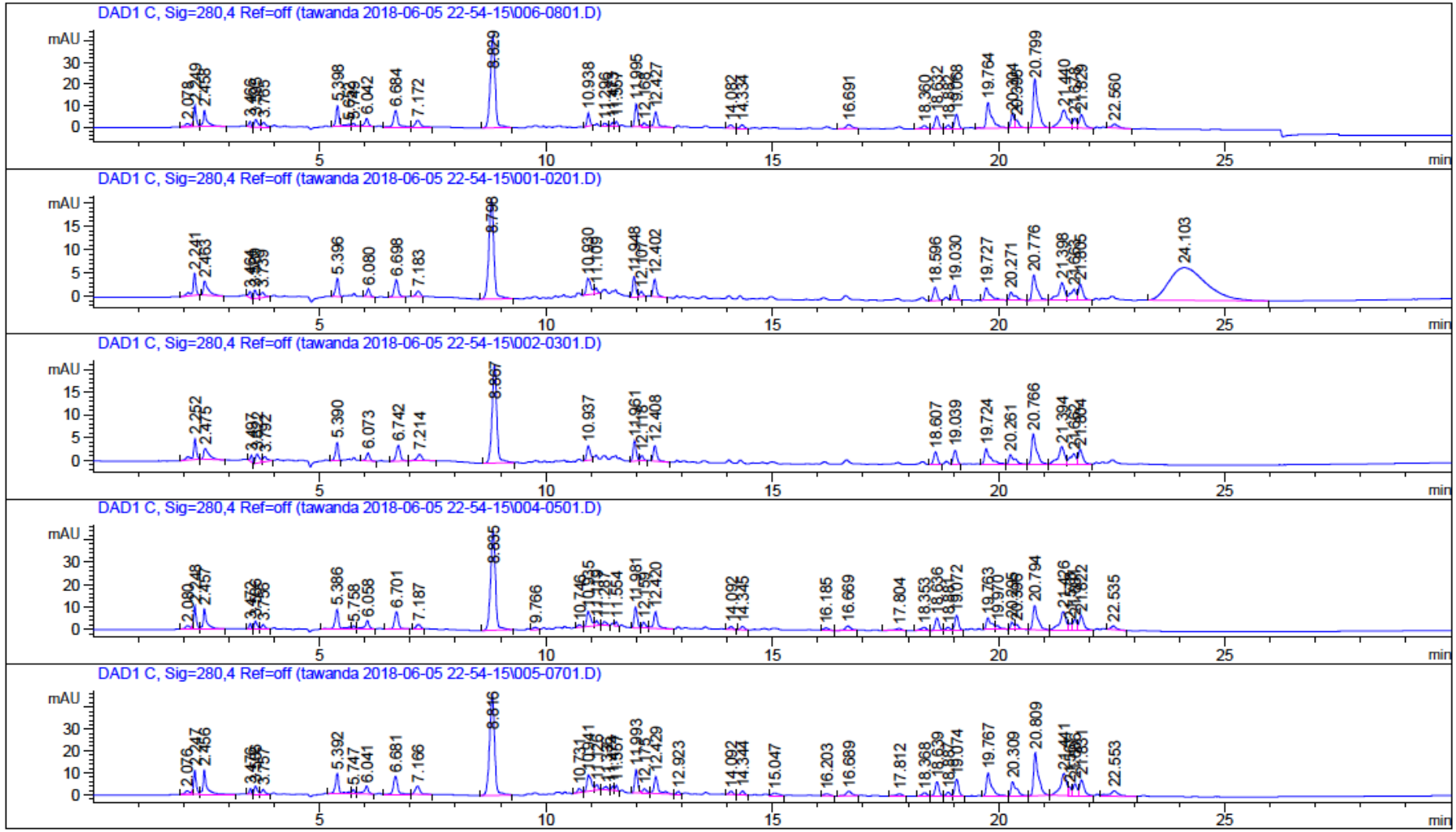


Figure 4 Chromatogram of sample 1rb1 bottles 1,2,3,4 and 5 at time 0

Figure 5 Figure 14 Chromatogram of sample 1rb1 bottles 1,2,3,4,5 and 6 at time 48 hours

Figure 6 Figure 14 Chromatogram of sample 1rb1 bottles 1,2,3,4, 5 and 6 at 14 days storage

Figure 7 Figure 14 Chromatogram of sample 1rb1 bottles 1,2,3,4,5 and 6 at 28 days storage

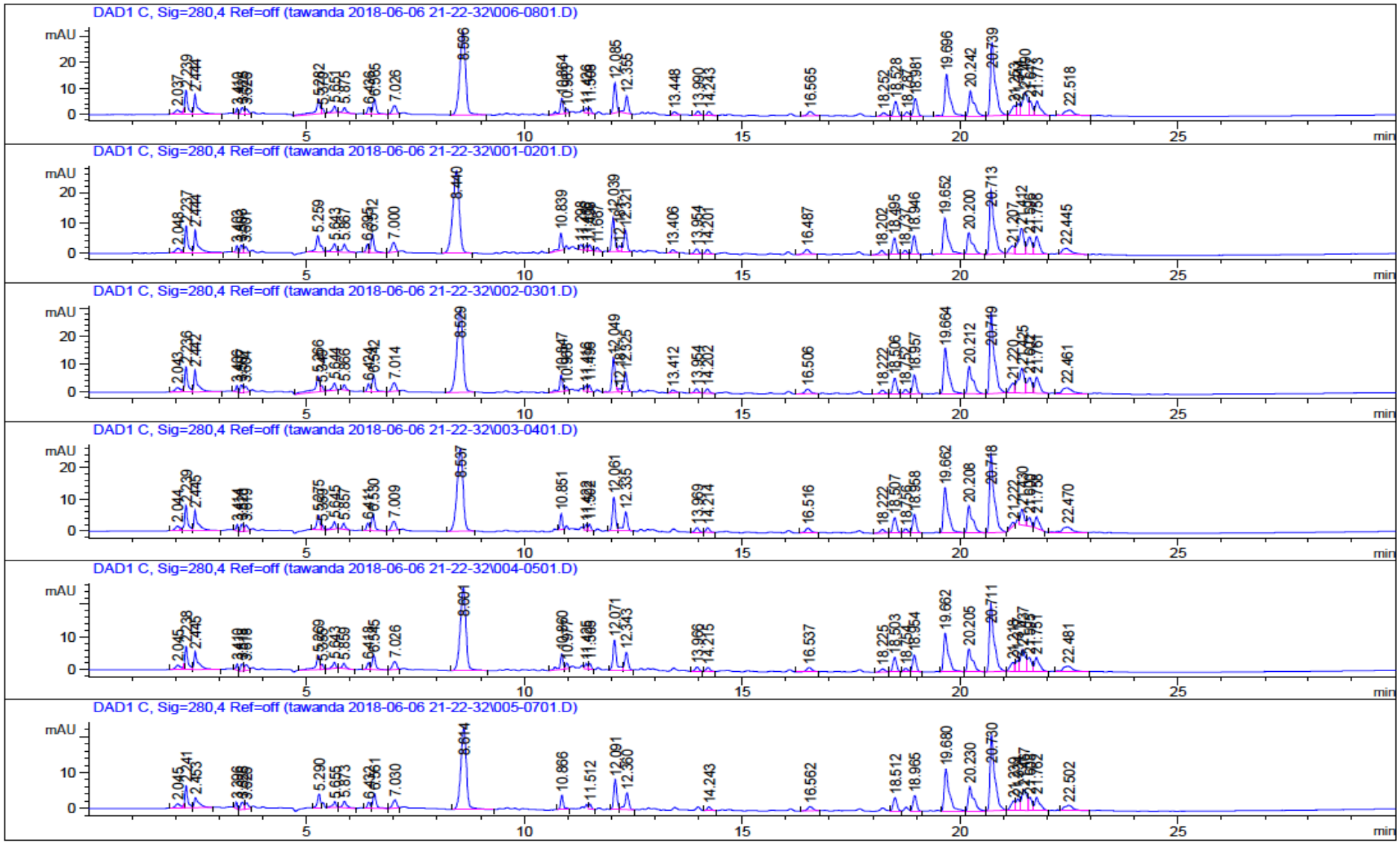


Figure 8 Figure 14 Chromatogram of sample 1rd4 bottles 1,2,3,4,5 and 6 at time 0

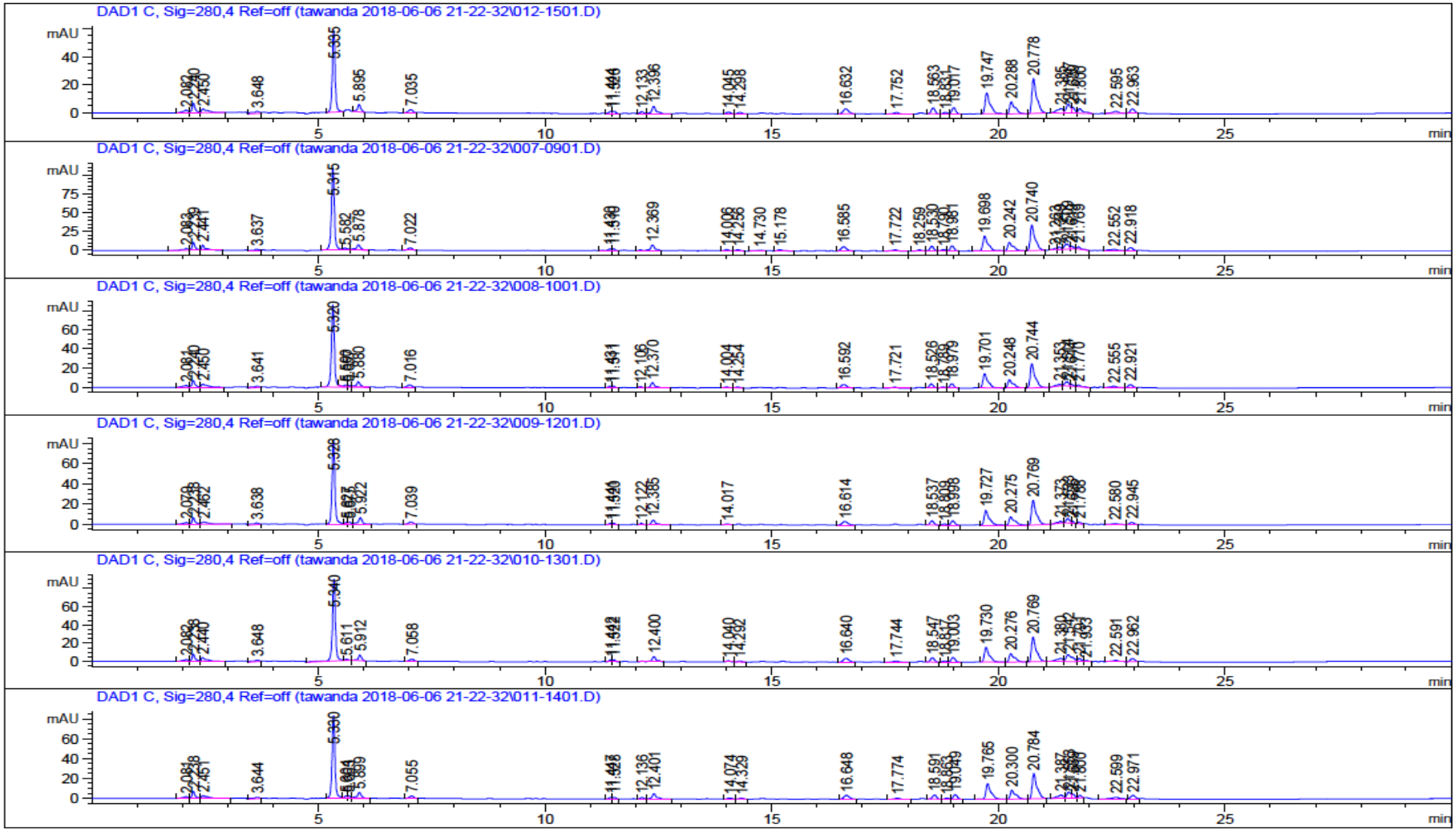


Figure 9 Figure 14 Chromatogram of sample 1rd4 bottles 1,2,3,4,5 and 6 at time 48 hours

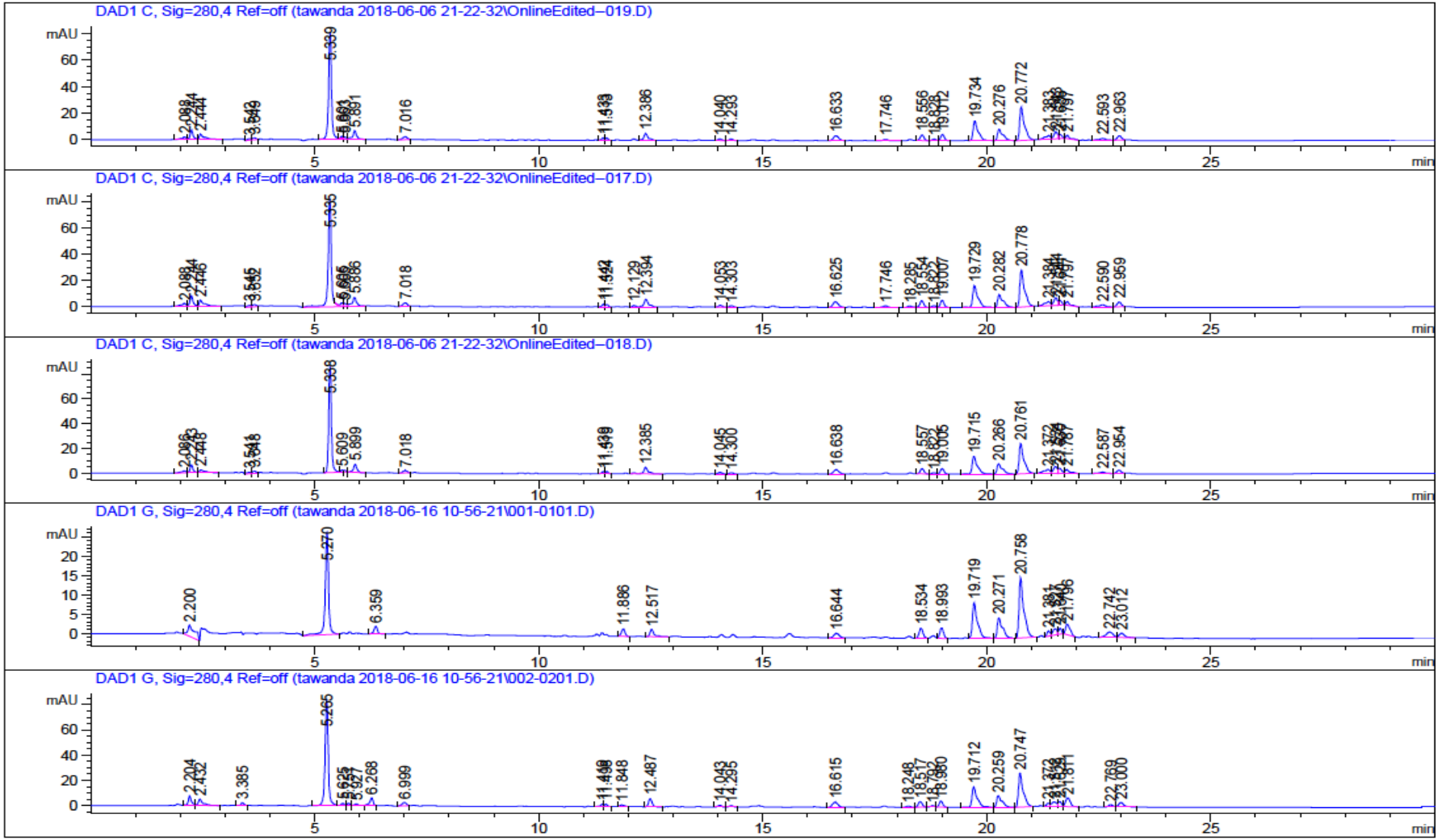


Figure 10 Figure 14 Chromatogram of sample 1rd4 bottles 1,2,3,4,5 and 6 at 14 days storage

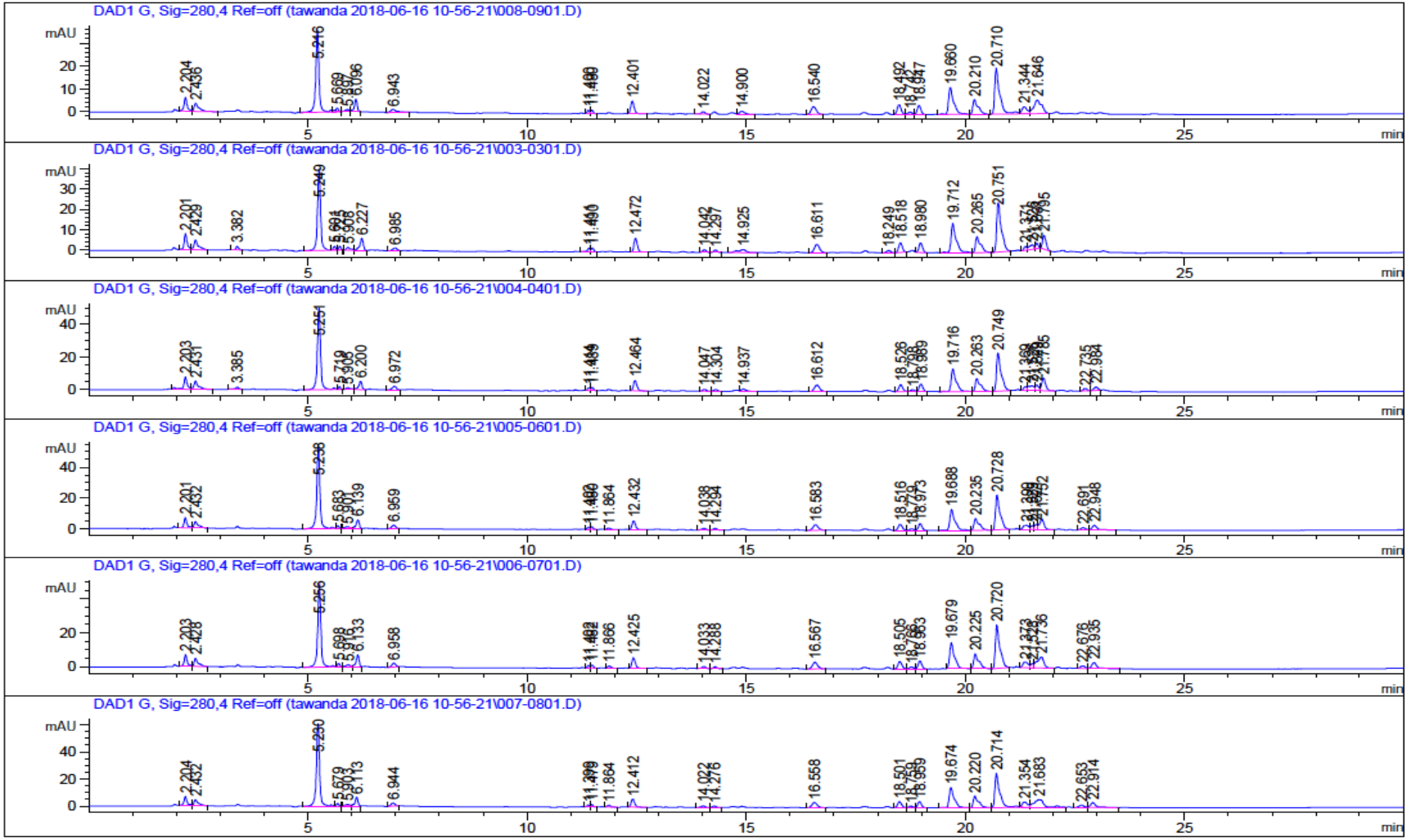


Figure 11 Figure 14 Chromatogram of sample 1rd4 bottles 1,2,3,4,5 and 6 at 28 days storage