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Physicochemical properties, metabolomic analysis, antioxidant and lipid-lowering activity of a functional beverage based on cocona (*Solanum sessiliflorum* Dunal)

[Propiedades fisicoquímicas, análisis metabolómico, actividad antioxidante e hipolipemiante de una bebida funcional a base de cocona (*Solanum sessiliflorum* Dunal]

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Abstract: The physicochemical, microbiological and metabolomics analysis, antioxidant and lipid-lowering effect, and shelf life prediction of a functional beverage based on cocona pulp of SRN9 ecotype was to carry out. According to the results obtained, the beverage complies with all the characteristics of the Peruvian technical standard for juices, nectars and fruit beverages NTP 203.110:2009 and is within the limits established by the sanitary technical standard NTS N° 071-MINSA/DIGESA-V.01, with a shelf-life period of 4 months and 1 day. The metabolome regarding bioactive compounds showed the presence of 30 compounds, including several glycosylated flavonols, two flavanols, and two spermidines. Likewise, showed a lipid-lowering effect statistically significant ($p < 0.05$) about the serum levels of total cholesterol and triglycerides, with a mean reduction of 41.52 mg/dL for total cholesterol levels and 130.80 mg/dL for triglyceride levels. This beverage could be an alternative for the treatment of atherosclerosis and prevention of cardiovascular diseases.

Keywords: Solanun sessiliflorum; Functional beverage; Triglycerides; HPLC-ESI-Orbitrap-MS; Antihyperlipidemic

Resumen: Se realizó el análisis fisicoquímico, microbiológico y metabolómico, efecto antioxidante e hipolipemiante, y vida útil de una bebida funcional a base de cocona ecotipo SRN9. De acuerdo a los resultados, la bebida cumple con las características de la norma técnica peruana para jugos, néctares y bebidas de frutas NTP 203.110:2009 y se encuentra dentro de los límites establecidos por la norma técnica sanitaria NTS N° 071-MINSA/DIGESA-V.01, con una vida útil de 4 meses y 1 día. Del perfil metabolómico se identificaron 30 compuestos, entre ellos varios flavonoles glicosilados, dos flavanoles y dos espermidinas. Asimismo, mostró un efecto hipolipemiante estadísticamente significativo ($p < 0,05$) sobre los niveles séricos de colesterol total y triglicéridos, con una reducción media de 41,52 mg/dL y de 130,80 mg/dL para los niveles de colesterol total y de triglicéridos, respectivamente. Esta bebida podría ser una alternativa para el tratamiento de la aterosclerosis y prevención de enfermedades cardiovasculares.

Palabras clave: Solanun sessiliflorum; Bebida funcional; Triglicéridos; HPLC-ESI-Orbitrap-MS; Antihiperlipidémico

INTRODUCTION

Cardiovascular diseases are the main causes of death in adults of both sexes, both in developed and developing countries. The most frequent cause of cardiovascular diseases is atherosclerosis, which is multifactorial and predisposes to myocardial infarction and cerebral thrombosis, among other diseases, in whose origin hyperlipidemia is involved as one of the risk factors (Smith *et al*., 2006; Rafieian-Kopaei *et al*., 2014). Hyperlipidemia is caused by a diet that contains too much cholesterol and fat, or when the body produces too much cholesterol and fat, or both; and usually causes no signs or symptoms. It is determined by a simple blood test that measures cholesterol and triglyceride levels. Too much "bad" cholesterol can build up in the arteries and, over time, can lead to heart disease or stroke. An elevated triglyceride level can also increase the accumulation of fat in the arteries and cause heart disease, especially in obese or diabetic people (Kreisberg & Reusch, 2005). Treatments with lipid-lowering drugs have shown a regulatory effect on blood cholesterol and triglyceride concentrations, however, not all patients tolerate this type of treatment. In some cases, these medications can cause adverse effects and also increase health care costs (Ewang-Emukowhate & Wierzbicki, 2013). In recent years, there has been growing consumption of the functional beverages as they provide a health benefit, beyond the basic nutritional functions, in disease prevention and treatment. Likewise, the optimization of the production and formulation of novel beverages and the use and processing of natural ingredients are one of the main directions of functional beverages (Corbo *et al*., 2014; Nazhand *et al*., 2020).

Cocona (*Solanum sessiliflorum* Dunal; Solanacea) is an Amazonian fruit widely consumed by the Amazonian people and is mainly used for preparation of jelly, juices, sauces, and marmalades, among other products. In traditional and popular Amazonian medicine, the fruit is used to treat several ailments such as burns, diabetes, and skin mycoses, and also to lower uric acid and cholesterol blood levels (Jiménez, 2018; Vargas-Arana *et al*., 2023). In the Peruvian Amazon there are several cocona ecotypes, each of which has its own difference based on its shape, size and production yield (Ospino *et al*., 2013). Previous studies in humans and experimental animals show that the cocona fruit, without specifying the ecotype, has a lipid-lowering effect (Pardo, 2004; Tocto-Chaquila *et al*., 2020). Recent studies carried out by our research group on five ecotypes of cocona fruit showed that this plant has a potential use to manage chronic diseases such as hyperlipidemia, especially the pulp of SRN9 ecotype, which presented the best results in antihyperlipidemic activity (Vargas-Arana *et al*., 2021).

In this context, this study aims to analyze the physicochemical, metabolomics characterization, metabolomic analysis, antioxidant and Lipid-lowering effect, and shelf life prediction of a functional beverage based on cocona pulp of SRN9 ecotype (Figure No. 1), sweetened with stevia, that naturally lowers cholesterol and triglyceride levels in the blood and intended for adults.

Figure No. 1
Beverage based on cocona pulp (*Solanum sessiliflorum*) of SRN9 ecotype
(Picture source: personal collection)

MATERIALS AND METHODS

Chemical

All chemicals and reagents used in the experiments were of analytical grade and purchased mostly from Merck (Lima, Peru), Sigma Aldrich Chem. (St. Louis, MO, USA) and Extrasynthèse (Genay, France).

Plant Material

The seed of cocona fruit (*Solanum sessiliflorum*) of SRN9 ecotype was obtained from the *ex situ* conservation gene bank of the Peruvian Amazon Research Institute, and cultivated in the Research and Production Center of Tulumayo of the National Agrarian University of the Jungle (09°06'20'' S and 75°54'15'' W, 565 meters altitude) Huanuco region, Peru.

Processing of the pulp and formulation of the beverage

The selected fruits were washed, brushed, disinfected using a 200 ppm sodium hypochlorite solution for 30 min and rinsed with water. The epicarp was then removed. Then thermal bleaching was carried out at 90°C for 20 min. The pulp was then extracted in a pulping machine removing the husks and seeds (Vargas-Arana *et al.*, 2021). A yield of 57.21% in cocona pulp SRN9 ecotype was obtained. The formulation of the cocona functional beverage was 1:1 (pulp:water), with 0.045% powdered stevia. These inputs were homogenized and brought to a temperature of 63°C to add 0.05% potassium sorbate for 5 minutes. After this time, the beverage was bottled in previously sterilized glass bottles and hermetically sealed. The pasteurization of the beverage took place at 75°C for 5 minutes, then were cooled and stored at room temperature until used for analyses.

Physicochemical analysis

Proximal Composition

AOAC (2005) procedures were used in all determinations. Moisture content was determined by oven drying the sample to a constant weight, crude protein content by the Kjeldahl method (N x 6.25), fiber content by gravimetric method after acidic hydrolysis of the samples, and ash content by incineration in a muffle furnace at 550 ± 15°C. Total lipids were extracted in a Soxhlet apparatus using petroleum ether as a solvent, and total carbohydrates were calculated as difference: 100 - (W_{water} + $W_{protein}$ + W_{fiber} + W_{fat} + W_{ash}). Results were expressed in %.

Mineral Analysis

For the mineral analysis, AOAC (2005) procedures were used. 10 mL of sample were incinerated in a muffle furnace at 500°C until a white ash was obtained. The ash in each case was boiled with 10 mL of 20% hydrochloric acid in a beaker, and then filtered into a 100 mL standard flask and made up to 100 mL with distilled deionized water. Levels of minerals iron (Fe), calcium (Ca), sodium (Na) and phosphorus (P) were determined from the resulting solution using atomic absorption spectroscopy (Varian AA240), previously calibrated with standard solutions containing known amounts of the minerals being determined with analytical grade reagents. Two types of flames were used: air-acetylene and nitrous oxide-acetylene, the latter only for calcium analysis. Monometallic hollow cathode lamps were used for each element analyzed. All analyzes were performed in triplicate.

pH

The pH of the sample was taken using a pH meter (WTW inolab® pH 7310 Benchtop Meter, Germany). To 20 mL of the functional beverage of cocona was taken to dip the calibrated electrode of the pH meter and the observations were recorded in triplicate for each sample.

Total Soluble Solids

The Total Soluble Solids (TSS) of the functional beverage was measured in triplicate using a calibrated hand refractometer (Erma hand refractometer, Japan), and the reading observed was expressed in Brix.

Titratable Acidity

The acidity of the functional beverage was carried out by the method 942.15 AOAC (2005). Titrate with 0.1 N NaOH solution, using 0.3 mL phenolphthalein for each 100 mL solution being titrated, and expressed as % citric acid after using the following formula [1]:

$$\text{Titratable acidity (\%)} = \frac{\text{Titre Value(mL) x N NaOH x Eq.Weight (Citric Acid)}}{\text{Sample Weight (g)}} \text{ x } 100 \quad [1]$$

Vitamin C

The Vitamin C estimation (Reduced Ascorbic Acid) was determined using the titrimetric method with 2,6-dichlorophenolindophenol reagent as per the Association of Official Analytical Chemists (AOAC, 2005). 10 mL of cocona functional beverage was mixed with 90 mL of 4% oxalic acid solution and filtered. From this, 5 mL of the filtrate was diluted to 15 mL with 4% oxalic acid solution and titrated against 0.02% of 2,6-dichlorophenolindophenol dye solution until the end point of light pink color persisted for 15 s. The factor of dye solution (2,6-dichlorophenolindophenol) was obtained through titration using 0.05% standard ascorbic acid solution. The Vitamin C calculated as per the following formula [2] and expressed in mg per 100 g.

Vitamin C content (mg/100 g) = (0.5 mg/V_1) x (V_2/15 mL) x (100 mL/wt. of Sample) x 100 [2]

V_1 and V_2 are the volumes of dye solution used for standard ascorbic acid titration and also for the cocona functional beverage sample, respectively.

Sugars

The sugars such as % reducing sugars, % non-reducing sugars and % total sugars in cocona functional beverage were determined as per the procedures of AOAC (2005) and calculated according to the following formulae [3], [4]:

Reducing sugars (%) = Factor x Dilution x Volume x 100/Weight of sample (%) Titer value [3]
Total Sugars (%) = Factor x Dilution x Volume x 100/Weight of sample (%) Titer value [4]

The % non-reducing sugars was obtained by difference between % total sugars and % reducing sugars.

Water activity (Aw)

Water activity (Aw) was measured using an AQUALAB PAWKIT portable 25°C dew point hygrometer.

HPLC MS analysis and MS parameters

The separation and identification of the compounds present in the cocona functional beverage (CFB) both in negative or positive ESI modes, were performed on a UHPLC-ESI-Orbitrap-MS system consisting of a Dionex Ultimate 3000 UHPLC system (Thermo Scientific) with a Q Exactive Plus (Thermo Scientific). A 50 mg of a freeze dried beverage sample (0.025 mbar, -55°C, 72 h), 2.5 mL of MeOH-H_2O (4:1, v/v) solution at 0.1% formic acid were added and extracted under ultrasound stirring for 20 min. The supernatant was separated in a 15 mL conical tube and the solid was extracted again under stirring with 2.5 mL of the same solution. The solutions were mixed in a conical tube and then centrifuged at 2500 rpm and 10°C for 20 min. The solution was filtered using a 0.45 µm syringe filter, and 5 µL was injected into the equipment. The chromatographic equipment consisted of a quaternary pump, an autosampler, a thermostated column compartment and a PDA, photodiode array detector. Elution was performed with a binary gradient system with eluent (A) 1% formic acid in the water, eluent (B) 1% formic acid in the acetonitrile and the gradient program (time (min), % A) was: (0.00 min, 95% A); (1.5 min, 95% A); (35.00 min, 5% A); (36.00, 5% A); (38.00 min, 95% A). Separation was carried out with a Luna© Omega C18 100 Å column (150 mm x 2.1 mm x 1.6 µm) with an injection temperature of 18°C and flow rate of 0.3 mL/min. ESI-Orbitrap-MS experiments were recorded in positive and negative ion mode, and the scan range was between 100 and 1500 m/z. The detection wavelengths were 240, 280, and 320 nm. Electrospray ionization (ESI) conditions consisting of a capillary temperature of 260°C, a capillary voltage of 2.5 KV, gas carrier: N_2 (Sheath gas flow rate: 48, Sweep gas flow rate: 2), gas heater temp: 280/280°C, S-lens RF level: 100. The experiments were performed in automatic MS/MS mode. The structural characterization of secondary metabolites was based on HR full MS, MS fragmentation patterns and search in the literature data.

Antioxidants Activity

Extraction of phenolics

1 mL of cocona functional beverage was placed in a centrifuge tube to which 4 mL methanol are added,

the mixture is vortexed for 15 min. It was then centrifuged at 5000 rpm for 15 min at 5°C. The supernatant (phenolic extract) was filtered and stored at -4°C until its respective analysis.

DPPH Scavenging Activity

The α, α-diphenyl-β-picrylhydrazyl (DPPH•) radical was assayed by the decolorization method (Brand-Williams *et al*., 1995). 100 µL of phenolic extract was filtered with a membrane filter (0.45 µm) and mixed with 3.9 mL of DPPH• radical solution (100 µM) dissolved in 80% methanol. The mixture was stirred vigorously and set in the dark for 30 minutes at 25°C. After that time, the absorbance at 517 nm was read in a UV-visible Cary60 spectrophotometer. The concentration of DPPH• in the reaction medium was obtained from a calibration curve by linear regression. The control consisted of 100 µL of 80% aqueous methanol and 3.9 mL of DPPH • solution (100 µM). The results were expressed in TEAC, that is, antioxidant activity equivalent to Trolox (µmol Trolox/mL of sample). The reference synthetic antioxidant Trolox, at a concentration of 5-30 µM in 80% methanol solution, was tested under the same conditions.

ABTS Bleaching Capacity

The ABTS assay was performed by bleaching of the 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS•+) radical cation as described by Re *et al*. (1999). The reaction was started with the addition of 1500 µL of an ABTS•+ solution in PBS buffer (0.70 ± 0.02 at $\lambda = 734$ nm) to 500 µL of the phenolic extract in a cuvette kept at 30°C. It was homogenized and allowed to react for 7 minutes, then the absorbance reading was made at a wavelength of 734 nm using a Cary60 UV-visible spectrophotometer. The results are expressed in TEAC (µmol Trolox/mL of sample). The calibration curve for TEAC was constructed using different concentrations of Trolox (4-14 µM) in PBS buffer solution under the same conditions.

Total Phenolic Content

Total phenolic compounds (TPC) were analyzed based on Velioglu *et al*. (1998) and Cobos *et al*. (2020), with some modifications. Briefly, 100 µL of the phenolic extract was mixed with 750 µL of the Folin-Ciocalteu reagent diluted in a 1/10 proportion of Milli-Q water. After 5 minutes in the dark, 750 µL of sodium bicarbonate (566 mM) was added to the mixture. The tubes were kept in the dark for 90 minutes at 30°C, then the absorbance was read at 725 nm using a Cary60 UV-visible spectrophotometer. Total phenolic content was determined based on the standard curve (from 10 to 100 µM) prepared from 3,4,5-trihydroxybenzoic acid (Gallic acid). The results are expressed as mg gallic acid per mL of sample.

Total Carotene Content

Total carotenoids were carried out according to Lee *et al*. (2001), with some modifications. 25 mL of cocona functional beverage was homogenized in a vortex with 50 mL of extracting solvent (hexane/acetone/ethanol, 50:25:25, v/v/v) and centrifuged for 30 min at 5000 rpm and 5°C. A saturated sodium bicarbonate solution was added and centrifuged again for 10 min. The supernatant from the hexane phase was extracted, rich in carotenoids, and its absorbance at 450 nm was determined using a Cary60 UV-visible spectrophotometer. The calculation of total carotenoids was performed by comparison with a calibration curve obtained with a certified β-carotene standard. The results were expressed as µg β-carotene per mL of sample.

Microbiological Analysis

1 mL of the cocona functional beverage was transferred to a test tube with 9 mL of diluent (1% Peptone Water) to obtain the 10-1 dilution, after which 1 mL was taken and placed in another tube to obtain the 10-2 solution. Subsequently, the sample was seeded in the mesophilic aerobic (Plate Count Agar), coliform (Violet Red Bile Agar), mold and yeast (Oxytetracycline Agar Gentamicin Yeast Extract Glucose) media, using casting technique in Petrifilm® plates with rehydratable dry films, where 1 mL of the dilution was seeded in the center of the circle. Subsequently, the inoculum was distributed using a plastic diffuser sheet and incubated in a horizontal position for 24-48h at 35°C for mesophilic aerobes and coliforms, and for 3-5 days at 25°C for molds and yeasts. The results of the aforementioned counts were expressed in colony-forming units per milliliters of sample (CFU/mL) (AOAC, 2005).

Determination of Shelf-Life

The prediction of the shelf-life of the cocona functional beverage was carried out according to Yoon *et al*. (2017). A slope, intercept, and correlation coefficient were calculated based on the Arrhenius Method model for kinetic degradation through linear regression analysis of physicochemical parameters: acidity and water activity; Microbiological: count of mesophilic aerobic microorganisms, enumeration of

total coliforms, count of molds and count of yeasts; Sensorial: appearance, color, smell and taste with a panel of 3 sensory judges (Table S1 in Supplementary Material), during the period of 20 days at temperature conditions of: 20°C, 30°C and 40ºC.

Lipid-lowering Activity

The effects of consuming the cocona functional beverage (CFB) were evaluated in 10 people aged 35 to 68 years of both genders (3 women and 7 men) without pharmacological treatment and with cholesterol and triglycerides levels above normal (cholesterol > 200 mg/dL, triglycerides > 150 mg/dL). All participants did so voluntarily under an informed consent letter. For this, each adult was administered a daily amount of 250 mL of the beverage for a period of 8 days, and they were told to eat a low-fat diet during treatment. The biochemical analysis of cholesterol and triglycerides was performed at two times, in basal conditions without treatment and after 4 days after the last intake, in order to evaluate the effects of the intake of the CFB as nutritional support for the control of hyperlipidemic. The respective biochemical analyzes were performed at the Clinical Analysis Laboratory of the National University of the Peruvian Amazon.

The protocol of this study was approved by the Institutional Research Ethics Committee of the National University of the Peruvian Amazon (protocol number PI-062-27/12/22-CIEI-UNAP).

Statistical Analysis

The statistical analysis was carried out using SigmaPlot 11.0 software. The experimental were replicated three times. The values obtained are expressed as the mean ± standard deviation. The lipid-lowering activity the results were analyzed by applying t-student for correlated samples (before and after design), with a significance level of the *p* value < 0.05.

RESULTS AND DISCUSSION

Physicochemical Analysis

Physicochemical characteristics of cocona functional beverage (CFB) are presented in Table No. 1. According to the Peruvian technical standard NTP 203.110:2009 for fruit juices, nectars and beverages (INDECOPI, 2009), the pH for beverages should be less than 4.5, therefore the pH of CFB is within the range. Likewise, the standard establishes that the specification range for total soluble solids (°Brix) is 12, and here we report a lower value than that established by the standard because stevia was used as a sweetener instead of sucrose (Salar *et al*., 2020). The proximal analysis and the mineral content maintain the relationship with the cocona pulp SRN9 ecotype, taking into account the dilution with which CFB was made (Vargas-Arana *et al*., 2021). The protein content in CFB was higher than that reported by Quispe-Herrera *et al*. (2022), for nectar of cocona enriched with quinoa (*Chenopodium quinoa*), but lower in vitamin C content (11.6 mg/100 g). The titratable acidity value of CFB was 1.18%, an expected value because low values have also been reported for *Solanum sessiliflorum* pulp (Andrade *et al*., 2016), which gives us a drink with a characteristic acid flavor of the fruit. The value of reducing sugars of CFB was lower than that reported for the fresh pulp (2.28%) and reconstituted freeze-dried pulp (1.46%) of cocona, and this variance may be due to the heat treatment received by the fruit for the preparation of the beverage (Natividad-Marín & Cáceres-Paredes, 2013).

HPLC-ESI-Orbitrap-MS analyses

The compounds of cocona functional beverage were identified with the aid of PDA analysis and high resolution Orbitrap mass spectrometry (HPLC-ESI-Orbitrap-MS), some 30 compounds were detected and identified (Table No. 2). The chromatogram is depicted in Figure No. 2 and detailed identification is shown below. Several compounds were already found in extracts of five cocona ecotypes fruits NMA1, SRN9, CD1, CTR, UNT2 (Vargas-Arana *et al*., 2021).

Carbohydrates

Peaks 1, 2 and 3 were identified as gluconic, citric and isocitric acid respectively. While peak 4 as quinic acid and peak 5 as the sugar rolinose and peak 13 as Diterbutyldicarbonate ($C_{10}H_{18}O_5$).

Table No. 1
Physicochemical properties of CFB

Parameters	Value
Humidity %	94.72 ± 0.14
Total solids %	5.28 ± 0.14
Ashes %	0.32 ± 0.01
Total lipids %	0.55 ± 0.02
Crude protein %	0.46 ± 0.02
Crude fiber %	2.09 ± 0.10
Carbohydrates %	1.86
Calories	20.91
Density (g/mL)	1.03 ± 0.04
pH	3.81 ± 0.17
TSS (°Brix)	3.27 ± 0.08
Titratable Acidity (%)	1.18 ± 0.03
Vitamin C (mg/100 g)	2.74 ± 0.05
Fe (mg/100 g)	0.09 ± 0.00
Na (mg/100 g)	1.10 ± 0.02
Ca (mg/100 g)	2.78 ± 0.13
P (mg/100 g)	22.9 ± 0.85
Reducing sugar (%)	0.60 ± 0.02
Non-reducing sugar (%)	0.66 ± 0.03
Total sugars (%)	1.26 ± 0.03
Water activity	0.98 ± 0.04

Phenolic acids

Peaks 6 with an anion at m/z: 515.11952 was identified as di coumaroyl quinic acid (3,5 di C-QA, $C_{25}H_{23}O_{12}^-$) and peak 7 as the related compound chlorogenic acid (353.08820), both producing quinic acid daughter molecule. Peak 8 was identified as 1-*O*-Sinapoyl-glucose ($C_{17}H_{22}O_{10}$) and peak 9 as 3-O-Diglucosyl-4-methoxy-3-hydroxybenzoic acid ($C_{20}H_{27}O_{14}^-$), while peak 20 as protocatechuic acid 5-O-apiofuranosyl- glucose m/z: 461.13023 ($C_{19}H_{26}O_{13}$), peak 15 as apiosyl-glucosyl-hydroxybenzoate ($C_{18}H_{24}O_{12}$). Synapoyl glucose derivates are shown to exhibit anti-inflammatory, antioxidant, anticancer, antiglycemic, antimutagenic, neuroprotective, and antibacterial activities (Chen, 2016). Protocatechuic acid has been regarded as anticancer (Buskaran *et al*., 2021), while coumaroyl quinic acids have shown antibacterial activity, plus anti-carcinogenic, anti-inflammatory, cardioprotective, anti-obesity, anti-diabetic and antioxidant properties (Clifford *et al*., 2006; Plazas *et al*., 2013; Kuczkowiak *et al*., 2014), and also reduction of lipid hydroperoxide production (Liang & Kitts, 2015).

Flavonols and flavanols

Peak 10 and 11 were identified as the flavanols gallocatechin and its isomer ($C_{15}H_{13}O_7^-$), epigallocatechin-3-gallate was regarded as antidiabetic (Li *et al*., 2007) and have been shown to reduce plasma cholesterol levels and the rate of cholesterol absorption (Raederstorff *et al*., 2003). Peaks 12, 14, 16, 17, 18, 19, 21, 22 and 27 were identified as flavone glycosides naringenin-5,7-di-O-glucose, isorhamnetin-3, 7-di-O-glucose, rutin, isorhamnetin-3-O-rhamnose, kaempferol 3-*O*-rhamnose, isoquercitrin, quercetin 3-*O*-galactose, naringenin 7-*O*-rutinose and cirsimarin, while peaks 29 and 30 as aglycone flavonoids rhamnacin and naringenin. Flavonoids are peroxide quenchers acting as antioxidants, reducing ROS production, for instance kaempferol-3-*O*-L-rhamnoside is shown to have anticancer and antioxidant activity, by inhibition of AAPH induced oxidation in DNA and lipid peroxidation activity (Akter *et al*., 2022). While isorhamnetin-3, 7-di-O-glucose reduced serum levels of glucose and 5-(hydroxymethyl) furfural (5-HMF) in rats *in vivo* (Yokozawa *et al*., 2002), the O-

Glycoside quercetin or isorhamnetin derivatives showed a broad-spectrum of biological activities, including antiobesity and antidiabetic effects (Alizadeh & Ebrahimzadeh, 2022).

Spermidines

Peaks 24 and 26 with pseudomolecular ions at m/z: 638.30625 and 472.24414 were identified as N,N,N-tris (dihydrocaffeoyl) spermidine ($C_{34}H_{43}O_9N_3$) and N-Caffeoyl-N-(dihydrocaffeoyl) spermidine ($C_{25}H_{33}N_3O_6$), respectively, as reported previously in *Solanum sessiliflorum*. Polyamine spermidines can also act as antilipid agents since they reduce necrotic core formation and lipid accumulation inside the plaque (Michiels *et al*., 2016).

Fatty acids

Peak 28 with a M-H ion at m/z: 636.29358 was identified as the glucosilated fatty acid derivative Undecyl glucoside ($C_{29}H_{48}O_{15}^-$).

Isoflavones

Peaks 23 and 25 with pseudomolecular ions at m/z: 269.0595 and 431.09843 were devoided as genistein ($C_{15}H_{10}O_5$) and genistein 5-O-glucoside ($C_{21}H_{20}O_{10}$). Genistein showed lipid lowering effects modulating the plasma lipid indices in women with hyperlipidemias (Zhang & Chi, 2019).

Table No. 2
High resolution HPLC-ESI-Orbitrap-MS identification of metabolites in cocona functional beverage

Peak #	UV max	Tentative identification	Molecular Formula	Theoretical mass (*m/z*)	Measured mass (*m/z*)	Accuracy (δ ppm)	MS^n ions
1	-	Gluconic acid	$C_6H_{12}O_7$	195.05052	195.04993	3.03	177.0382
2	-	Citric acid	$C_6H_8O_7$	191.01944	191.01863	4.24	173.0080; 111.0078
3	-	Isocitric acid	$C_6H_8O_7$	191.01944	191.01947	3.25	111.00794
4	208	Quinic acid	$C_7H_{12}O_6$	191.05501	191.05573	2.34	127.04477
5	212	Rolinose	$C_{16}H_{30}O_8$	349.18569	349.18723	4.03	222.1120 163.0386
6		Di coumaroyl quinic acid (3,5 di C-QA)	$C_{25}H_{23}O_{12}^-$	515.11923	515.11952	-0.46	353.08772, (CA) 191.05712 (QA)
7	335	Chlorogenic acid	$C_{16}H_{18}O_9$	353.08631	353.08820	4.21	191.05574 179.0347 161.0240
8	329	1-*O*-Sinapoyl-glucoside	$C_{17}H_{22}O_{10}$	385.1142	385.11472	1.29	247.0612 223.0611 164.0704
9	325	3-*O*-Diglucosyl-4-methoxy-3-hydroxybenzoic acid	$C_{20}H_{27}O_{14}^-$	491.13953	491.14124	0.67	167.0342
10	208	Gallocatechin	$C_{15}H_{13}O_7^-$	305.07834	305.07861	-8.18	205.0504 190.0269
11	208	Epigallocatechin	$C_{15}H_{13}O_7^-$	305.07834	305.07825	-8.18	205.0504 190.0269
12	280	Naringenin-5,7-di-O-glucoside	$C_{27}H_{32}O_{15}$	595.16575	595.16772	3.32	271.06152, 153.01845,

							147.04482, 119.05661
13	-	Diterbutyldicarbonate	$C_{10}H_{18}O_5$	218.18160	218.18198	3.76	178.9982
14	254-354	Isorhamnetin-3, 7-di-O-glucoside	$C_{28}H_{32}O_{17}$	639.15423	639.15665	3.70	477.10131, 315.04953 (Isorh), 301.031032, 285.03743
15	240	Apiosyl-glucosyl-hydroxybenzoate	$C_{18}H_{24}O_{12}$	431.09802	431.09832	0.46	431.1196 299.0768
16	254-354	Rutin	$C_{27}H_{29}O_{16}^-$	609.09649	609.14709	3.27	300.02795, 189.0664
17	254-354	Isorhamnetin-3-O-Rhamnoside	$C_{22}H_{22}O_{11}$	461.10853	461.10842	-0.3	315.04632 (Isorh), 301.031041, 285.03765
18	265-366	Kaempferol 3-O-rhamnoside	$C_{21}H_{19}O_{10}^-$	431.09834	431.09726	2.75	285.0657 (K) 215.0698 146.0598 127.0389
19	254-354	Quercetin-3-O-glucoside Isoquercitrin	$C_{21}H_{20}O_{12}$	462.1022	462.1027 463.1056	1.08	300.0280 271.0251 255.0301 151.0032
20	280	Protocatechuic acid 5-O-apiofuranosyl-glucopyranoside	$C_{19}H_{26}O_{13}$	461.13012	461.13023	0.21	329.0872 167.0344 123.0443 108.0208
21	254-354	Quercetin 3-galactoside	$C_{21}H_{20}O_{12}$	463.12270	463.08893	3.94	350.20898, 300.02795, 151.00310
22	280	Naringenin 7-O-Rutinoside	$C_{27}H_{32}O_{14}$	579.17083	579.17291	3.59	271.06152, 151.00319
23	280	Genistein	$C_{15}H_{10}O_5$	269.0595	269.0598	0.72	253.0485
24	325	N,N,N-tris(dihydrocaffeoyl) spermidine	$C_{34}H_{43}O_9N_3$	638.30592	638.30625	0.46	474.2588 456.2484 293.1852 222.1120 165.0543 123.0439

25	280	Genistein 5-*O*-glucoside	$C_{21}H_{20}O_{10}$	431.0980	431.09843	0.46	414.3355 271.0595 253.0485
26	330	N-caffeoyl-N-dihydrocaffeoyl spermidine	$C_{25}H_{33}N_3O_6$	472.24393	472.24414	0.42	455.2163 310.2118
27	-	Cirsimarin	$C_{23}H_{24}O_{11}$	475.12668	475.12524	4.36	271.06143, 152.07117
28	-	Derivate undecyl glucoside	$C_{29}H_{48}O_{15}^-$	636.29743	636.29358	-8.56	152.0108
29		Rhamnacin	$C_{17}H_{14}O_7$	329.06574	329.0666	-2.91	315.0462, (M-CH3), 277.1075, 300.05554, 151.0020, 256.03405
30	225	Naringenin	$C_{15}H_{12}O_5$	271.06010	271.06155	5.36	293.1852 72.0813

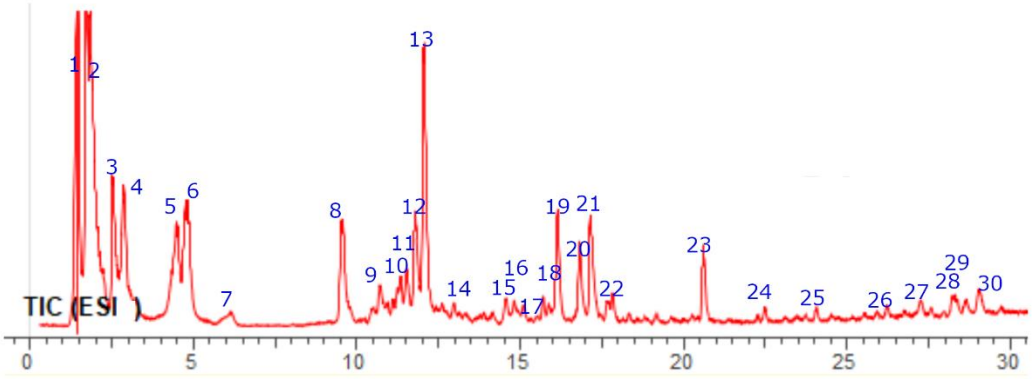

Figure No. 2
High resolution HPLC-PDA-ESI-OT-MS chromatogram of cocona functional beverage

Antioxidants activity, total carotenes and total polyphenols content

Antioxidant capacities of the cocona functional beverage (CFB) were evaluated by the trapping of DPPH and ABTS, and expressed as µmol Trolox/g sample. In addition, total phenolic content by the Folin-Ciocalteu method plus the total carotenes was evaluated. The antioxidant activity by the DPPH and ABTS assays were 1.84 ± 0.06 and 2.42 ± 0.11 µmol Trolox/mL respectively, values higher than those reported by Abdullakasim *et al*. (2007), for mango, orange and pineapple juices (18.9 ± 4.3, 12.3 ± 2.0 and 9.2 ± 0.4 µmol Trolox/100 mL) using the DPPH assay. Likewise, these values are comparable with those report for cocona nectar enriched with quinoa (*Chenopodium quinoa*), DPPH 3.49 µmol Trolox/g and ABTS 4.06 µmol Trolox/g (Quispe-Herrera *et al*., 2022), but lower values than those reported by Londoño *et al*. (2013), for coffee beverages, whose values range from 13.31 to 36.91 µmol trolox/mL for DPPH, and from 18.96 to 52.02 µmol Trolox/mL for ABTS. The total phenolic content of the CFB was 2.82 ± 0.08 mg GAE/mL, a value higher than those reported for different Thai health beverages, such as

bael fruit beverage with 83.89 ± 37.6 mg GAE/100 mL (Abdullakasim *et al*., 2007), but a value comparable to that reported pomegranate juice, which contained 3874.42 ± 14.5 µg GAE/ mL (Castro-López *et al*., 2016). The total carotenoid content of the CFB was 7.31 ± 0.38 µg β-carotene/mL, which is relatively similar to the results found for commercial Pomegranate juice (Castro-López *et al*., 2016). However, this is a lower value than that reported by Quintero *et al*. (2020), for a beverage formulated with aguaje pulp (*Mauritia flexuosa*) and cocona pulp with 48.21 ± 3.42 mg β-carotene/ 100 g sample. This high difference is due to the fact that the aguaje is a carotene-rich palm fruit (Pacheco Santos, 2005). According to the data obtained, the CFB values were lower compared to the cocona pulp dried of the SRN9 ecotype (Vargas-Arana *et al.*, 2021). This difference is related to the decrease in the concentration of the cocona pulp in the preparation of the beverage, without neglecting the degradation of micronutrients during processing, principally due to heating during pasteurization (Ordoñez-Santos & Martínez-Girón, 2020).

Microbiological Analysis and Shelf-Life

The microbiological analysis of the cocona functional beverage (CFB) is shown in Table No. 2, which indicates that the process carried out to obtain the CFB ensures a beverage free of microorganisms and suitable for human consumption according to the sanitary norm that establishes the microbiological criteria of sanitary quality and safety for food and beverages for human consumption NTS N° 071-MINSA/DIGESA-V.01 (DIGESA, 2023).

Table No. 3
Microbiological analysis of the cocona functional beverage

Parameters	Unit	Result
Mesophilic aerobic count	CFU/mL	<10*
Mold count	CFU/mL	<1*
Yeast count	CFU/mL	<1*
Enumeration of total coliforms	MPN/mL	<3

***Estimated count; CFU/mL: colony forming units per milliliters; MPN/mL: most probable number per milliliters**

The evaluation of shelf-life through the Arrhenius method was carried out. The CFB was subjected to temperature conditions of 20°C, 30°C and 40°C, for 20 days. The data and results are detailed in Supplementary Material (Table S2-S4, Figure S1-S4). The sensory characteristics of the product did not show variation during the sample analysis period at any of the three temperatures evaluated. Regarding the microbial population, it remains constant and no significant change was evidenced during the evaluation period of the product. The reaction rate of the study had a value of K = 0.000302. The cut-off point was determined by the water activity (Aw) on day 20 above the limits established for the CFB, at a temperature of 40ºC. As a result, it was obtained that CFB, under storage conditions at room temperature (25°C), has a shelf-life of 121.6458377 days, equivalent to 4 months and 1 day. This period is longer than reported by Tiencheu *et al*. (2021), for thirty juice formulations based on orange or lemon fruits, where all the juices with preservative lasted four weeks at room temperature. But period similar to that reported for a fermented beverage made from sugar cane juice (Olivares Muñoz & Vera Julón, 2019). All results obtained are within the limits established by the NTS N° 071-MINSA/DIGESA-V.01 (DIGESA, 2023). Next the sanitary registry was processed and it was approved with code P2350221N OAISDE by the General Directorate of Environmental Health of the Ministry of Health of Peru, where it is authorized for consumption and sale in the national market.

Lipid-lowering Activity

The results obtained from the evaluation of the lipid-lowering activity of the cocona functional beverages (CFB) are shown in Figure No. 3. The extent of cholesterol lowering in response to fiber consumption is variable, depending on several factors including the type of dietary fiber, nature of the background diet, and amount of fiber consumed. One of the actions of fibers in the intestine is to reduce dietary fat and cholesterol uptake interfering with key physiological events in the cholesterol absorptive process, in addition, the reduction in hepatic free cholesterol concentrations after fiber consumption is proposed to alter hepatic lipoprotein metabolism, reduction in hepatic apolipoprotein B and the formation of large

triglyceride-rich, cholesteryl-ester-depleted LDL (VLDL) particles Rideout *et al*. (2008). The increase in the fecal loss of bile acids and a reduction in the enterohepatic bile acid pool size may stimulate the liver to produce more bile acids from cholesterol, thus reducing hepatic free cholesterol concentrations. For the period of administration of the CFB, the traditional use of cocona fruit extract was taken as a reference. People mention that consumption of the extract is generally done in an approximate volume of 50 mL (with blending and straining 3 cocona fruits) and for a maximum of 8 days. Cardioprotective benefits and hypolipidemic effects associated with dietary fiber consumption are of paramount importance Rideout *et al.* (2008). In the case of cocona fruits beverage its amount of fiber (2.09 g, Table No. 1), plus its chemical constituents could be responsible for the reduction plasma cholesterol concentrations. Taking in consideration the consumption protocol, and after carrying out the comparative study of the concentrations of total cholesterol, we applied the t-student test to compare the mean concentrations of total cholesterol, before (239.90 ± 29.46 mg/dL) and after (198.39 ± 21.33 mg/dL) the CFB administration, which shows a value of t = 3.610 with a *p* value = 0.002. This confirms that the cholesterol reduction produced by CFB is statistically significant. The CFB produced a mean reduction of 41.52 mg/dL in total cholesterol levels, a comparable value (44.55 mg/dL) to that reported by Pardo (2004), for an extract of cocona pulp (40 mL) obtained at the moment and administered during 3 consecutive days. Yet, this value better than what was reported by Dolores *et al*. (2018), for a beverage elaborated from the pulp of *Physalisis angulata*, *Passiflora edulis* and *Ananas comosus*, where the administration of this beverage for three months only obtained a reduction of 10.3 mg/dL, and the initial and final comparison of cholesterol mean values indicated a statistically insignificant difference ($p>0.05$). Regarding the effect of CFB on triglyceride levels, this was better than for total cholesterol, and in all cases a decrease in the concentration of triglyceride levels was observed. A comparison of mean concentrations before (336.20 ± 136.60 mg/dL) and after (205.40 ± 72.88 mg/dL) was statistically significant with a value of t = 2.672 and *p* value = 0.016, applying the t-student test. There was a mean reduction of 130.80 mg/dL, a value higher than that reported by Pardo (2004) for a cocona pulp extract (50.84 mg/dL) and Dolores *et al*. (2018), for a beverage elaborated from the pulp of three fruits (40.70 mg/dL).

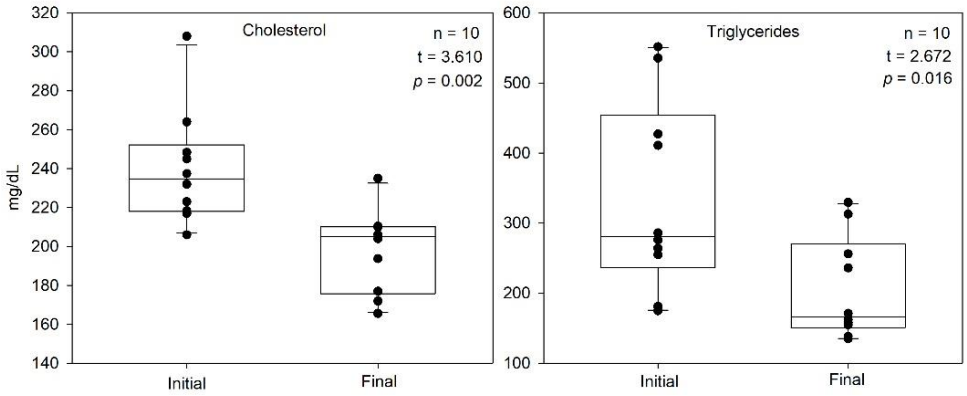

Figure No. 3
Cholesterol and triglyceride levels before and after CFB administration

CONCLUSIONS

In this study, a functional beverage was elaborated from the cocona pulp of the SRN9 ecotype, which guarantees the traceability of the product. The physicochemical and microbiological character-rization, antioxidant and lipid-lowering activity, metabolomic analysis and the determination of shelf-life accelerated by Arrhenius method was evaluated. According to the physicochemical tests, the CFB is within the permitted ranges and complies with all the characteristics of the Peruvian technical standard for juices, nectars and fruit beverages NTP 203.110:2009, with a pH = 3.81 ± 0.17 and TSS (°Brix) = 3.27 ± 0.08. The metabolome regarding bioactive phenolic compounds showed the presence of 30 compounds by HPLC-ESI-Orbitrap-MS analysis, including several glycosylated flavonols, two flavanols, and two spermidines in the beverage.

The antioxidant activity and the total carotenes content is higher than that reported for other fruit juices, and these values are related to that reported for the cocona pulp of the SRN9 ecotype, taking into account the decrease in pulp concentration when prepare the beverage. Likewise, the microbiological analysis and shelf-life results are within the limits established by the sanitary technical standard NTS N° 071-MINSA/DIGESA-V.01, with a duration at room temperature of 4 months and 1 day, which indicates that the CFB is suitable for human consumption. The CFB showed a lipid-lowering effect statistically significant ($p<0.05$) about the serum levels of total cholesterol and triglycerides, especially in the latter, so it could be an effective and safe alternative for the treatment of atherosclerosis and prevention of cardiovascular diseases. Therefore, it is necessary to carry out clinical trials with positive controls made up of authorized lipid-lowering drugs and a larger sample size to determine the magnitude of the efficacy of the functional beverage. In addition, this product with good diffusion can become a good biobusiness opportunity.

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SUPPLEMENTARY MATERIAL

Physicochemical properties, metabolomic analysis, antioxidant and lipid-lowering activity of a functional beverage based on cocona (*Solanum sessiliflorum* Dunal)

[Propiedades fisicoquímicas, análisis metabolómico, actividad antioxidante e hipolipemiante de una bebida functional a base de cocona (*Solanum sessiliflorum* Dunal)]

Determination of Accelerated Shelf Life - Arrhenius Method: Model for Kinetic Degradation

This study was conducted under controlled storage conditions of 20°C, 30°C and 40°C for 20 days. Therefore, the Arrhenius model describes the relationship of the reaction rate constant with temperature, using the measurement of physicochemical and sensory parameters and microbiological tests. This dependence is shown in the equation:

$$K(T)=KoExp\ (-Ea/(R.T)) \quad \ldots\ldots (1)$$

Where:
Ea: Activation Energy (cal/mol).
R: gas law constant (1.98 cal/mol)
T: Temperature in °K
K (T): Reaction rate constant at temperature T

Linearizing to a first order equation to obtain the VALUE of K:

$$Ln(K)=Ln(Ko)-Ea/R\ (1/T) \quad \ldots\ldots (2)$$

Table S1 - Response scales for sensory testing

Sensory characteristic	3: Desirable quality	2: Tolerable quality	1: Negative quality
Aspect	Homogeneous, lump-free, free of foreign matter	Slight loss of homogeneity, very slight presence of fine lumps that dissolve under pressure	Inhomogeneous, moldy, lumpy, with sediment, presence of foreign matter, live and dead insects
Color	According to the nature of the product	Slightly opaque colors, not very bright, slightly uneven shade	Presence of strange colors, due to mold (dark greys, greens, browns, etc.) of various shades
Smell	According to the nature of the product, free of extreme odors, rancidity, etc.	Low odor but free of extraneous odors	Sour, rancid, musty, musty, putrid, putrid or other foreign odors
Flavor	In accordance with the nature of the product, free of off-flavors, rancidity, among others.	Intense flavor but free of off-flavors	Sour, rancid, musty, musty, putrid, putrid or other off-flavors

Table S2. Microbiological tests at 20 °C, 30 °C and 40 °C

Temperature 20 °C				
Day	Aerobic Mesophilic (CFU/mL)	Coliforms (MPN/mL)	Molds (CFU/mL)	Yeast (CFU/mL)
8	<10	<3	<10	<10
10	<10	<3	<10	<10
12	<10	<3	<10	<10
14	<10	<3	<10	<10
16	<10	<3	<10	<10
18	<10	<3	<10	<10
20	<10	<3	<10	<10
Temperature 30 °C				
Day	Aerobic Mesophilic (CFU/mL)	Coliforms (MPN/mL)	Molds (CFU/mL)	Yeast (CFU/mL)
8	<10	<3	<10	<10
10	<10	<3	<10	<10
12	<10	<3	<10	<10
14	<10	<3	<10	<10
16	<10	<3	<10	<10
18	<10	<3	<10	<10
20	<10	<3	<10	<10
Temperature 40 °C				
Day	Aerobic Mesophilic (CFU/mL)	Coliforms (MPN/mL)	Molds (CFU/mL)	Yeast (CFU/mL)
8	<10	<3	<10	<10
10	<10	<3	<10	<10
12	<10	<3	<10	<10
14	<10	<3	<10	<10
16	<10	<3	<10	<10
18	<10	<3	<10	<10
20	<10	<3	<10	<10

CFU/g: Colony forming units per milliliters; MPN/mL: Most Probable Number per milliliters

Table S3. Physical-chemical test at 20 °C, 30 °C and 40 °C

Temperature 20 °C			
Day	Acidity (%)	Aw	LN (Aw)
8	1.16	0.97512	-0.02519
10	1.16	0.97534	-0.02497
12	1.17	0.97575	-0.02455
14	1.17	0.97617	-0.02412
16	1.17	0.97643	-0.02385
18	1.18	0.97663	-0.02365
20	1.18	0.97697	-0.0233
Temperature 30 °C			
Day	Acidity (%)	Aw	LN (Aw)
8	1.16	0.97525	-0.02506
10	1.17	0.97584	-0.02446
12	1.17	0.97624	-0.02405
14	1.18	0.97721	-0.02305
16	1.18	0.97763	-0.02262
18	1.18	0.97794	-0.02231
20	1.18	0.97812	-0.02212
Temperature 40 °C			
Day	Acidity (%)	Aw	LN (Aw)
8	1.16	0.97531	-0.02500
10	1.17	0.97654	-0.02374
12	1.18	0.97854	-0.02169
14	1.19	0.98113	-0.01905
16	1.21	0.98412	-0.01601
18	1.23	0.98854	-0.01153
20	1.24	0.99214	-0.00789

Table S4. Sensory Tests at 20 °C, 30 °C and 40 °C

Temperature 20 °C				
Day	Aspect	Color	Smell	Flavor
8	3	3	3	3
10	3	3	3	3
12	3	3	3	3
14	3	3	3	3
16	3	3	3	3
18	3	3	3	3
20	3	3	3	3
Temperature 30 °C				
Day	Aspect	Color	Smell	Flavor
8	3	3	3	3
10	3	3	3	3
12	3	3	3	3
14	3	3	3	3
16	3	3	3	3
18	3	3	3	3
20	3	3	3	3
Temperature 40 °C				
Day	Aspect	Color	Smell	Flavor
8	3	3	3	3
10	3	3	3	3
12	3	3	3	3
14	3	3	3	3
16	3	3	3	3
18	3	3	3	3
20	3	3	3	3

Experimental behavior at three different temperatures for the cocona functional beverage product

Y = A + BX	
Equation	Value
Y	Y
X	X
A	-0.0265
B	0.0002

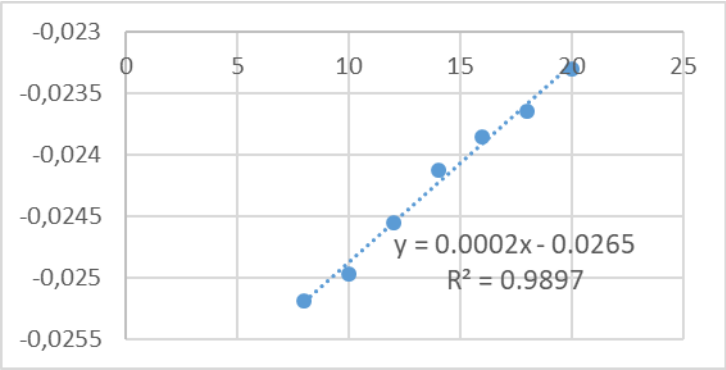

Figure S1. Water activity (Aw) results at 20° C

Y = A + BX	
Equation	Value
Y	Y
X	X
A	-0.027
B	0.0003

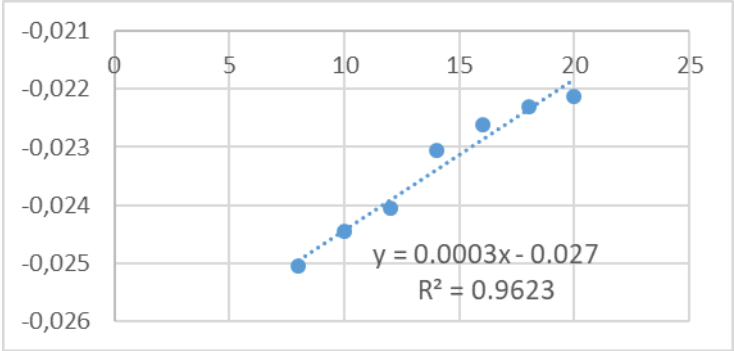

Figure S2. Water activity (Aw) results at 30 °C

Y = A + BX	
Equation	Value
Y	Y
X	X
A	-0.0382
B	0.0015

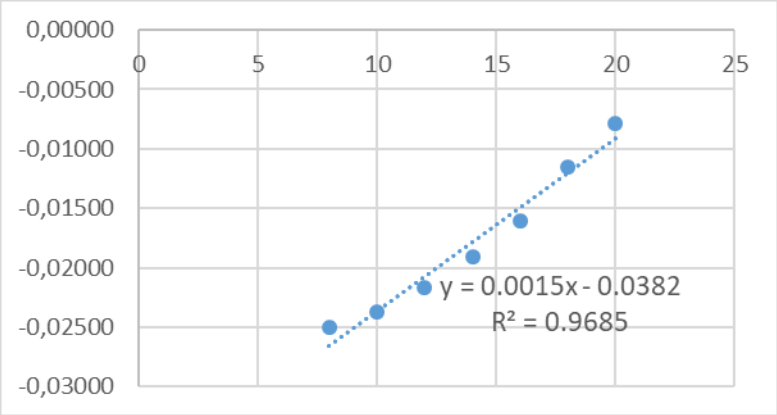

Figure S3. Water activity (Aw) results at 40 °C

Main Regression

T (°C)	T (K)	1/T	K(n=1)	Ln(K)
20	293	0.0034	0.0005748	-7.4615
30	303	0.0033	0.0022477	-6.0978
40	313	0.0032	0.0029721	-5.8185

Interpolation Estimation
(Acceleration Factor)

$$\frac{\partial A}{\partial t} = \pm K \mathrm{x} [A]^n$$

Where:
A: Quality Attribute
K: Reaction Rate Constant
T: Time

The cut-off point of the study was Aw, as it exceeded the maximum permissible levels (0.99) at a temperature of 40 °C (see results in the Table S3, T = 40 °C).

By linear regression the following results are obtained

Y = A + BX	
Equation	Value
Y	-8.732958264
X	0.003412969
A	25.253
B	-10020
K	0.000302
t	121.6458377

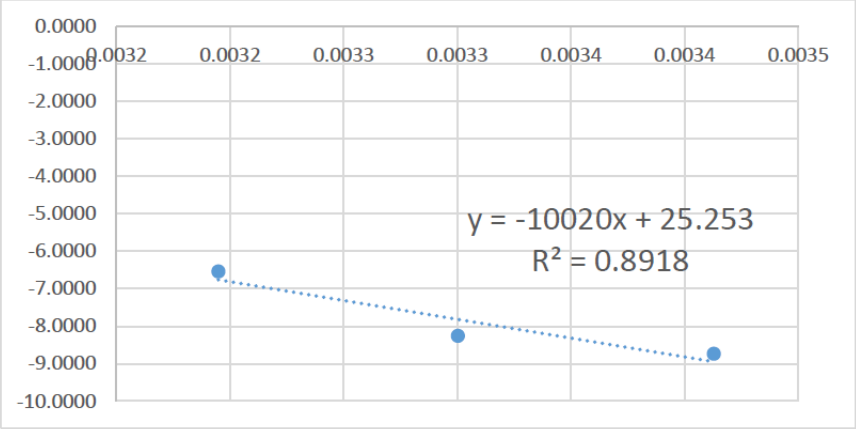

Figure S4. Linear regression result

Having the correlation values (R^2) close to 1, it can be ensured that the evaluation study is reliable

121.6458377 days = 4 months and 1 day