International Journal of Food Science and Nutrition
www.foodsciencejournal.com
ISSN: 2455-4898
Received: 25-07-2021, Accepted: 10-08-2021, Published: 26-08-2021
Volume 6, Issue 4, 2021, Page No. 127-133

Effect of adding cocoa powder on the quality of milk and its antioxidant potential

Samira Lagha-Benamrouche[1, 2*], Khaled Boudjema[1, 3], Djeziri Mourad[3, 4], Rezgui Walid[3, 4]

[1] Department of Biology, Faculty of Sciences, M' Hamed Bougara University, Boumerdès, Algeria

[2] Research Laboratory Soft Technology, Valorization, Physico chemistry of Biological Material and Biodiversity, Faculty of Sciences, UMBB University, Boumerdès, Algeria

[3] Research Laboratory in Food Technology, Faculty of technology, University M'hamed Bougara of Boumerdès, Algeria

[4] Center for scientific and technical research in Physico-chemical analysis (CRAPC) BP 384 Bouismail, Tipaza, Algeria

Abstract

The objective of our work is to study the influence of the addition of cocoa powder on the quality of the milk and its Antioxidant potential, by analyzing the chemical composition and the antioxidant power of two types of UHT milk (chocolate and plain) and monitoring their stability during storage (30°C/120 days and 55°C/30 days). The results show that cocoa powder is very rich in polyphenols compounds and has a strong antioxidant potential. Its addition to milk increases its antioxidant potential. Monitoring the quality of milk during storage shows on one hand no variation in the physicochemical parameters, the polyphenols and the fat contents for the two types of milk. On the other hand, the heat and the duration of storage reduce the contents of sugars, proteins and ash. The results show also that the antioxidant potential is stable for chocolate milk, whereas it significantly decreases during the two storages for plain milk. The UHT treatment associated with the presence of antioxidant agents makes the product more stable even at high temperatures and long storage times.

Keywords: antioxidant activity, chocolate milk, cocoa powder, plain milk, Stability, UHT

Introduction

Milk is a product of high nutritional value. It is one of the few foods to suit all age groups (infant, child, adolescent, adult, elderly) who consume it as is in liquid state (fresh milk) or in the form of derived products (fermented milks, cheeses, etc.) (Yakhlef 2010) [47]. The production of dairy products uses technologies intended to stabilize and sterilize products or to manufacture derived products, valued for their sensory and techno-functional properties (Debry 2001) [42]. The UHT (Ultra High Temperature) treatment is considered to be a treatment of choice which allows the total destruction of the milk microflora and the inhibition of degradation enzymes, while maintaining the organoleptic and nutritional qualities of the milk (Vigniola 2002) [45]. The innovations proposed in the field of milk have recently focused on the aromas and production of flavored milks enriched with vitamins which are good for health and provide energy. Among flavored milks, chocolate UHT milk is the most consumed milk drink. Cocoa powder, obtained after fermentation and roasting of the kernel of cocoa beans (*Theobroma cacao* subsp. *lineaus*), constitutes a reservoir of phenolic compounds. It stands out among foods as being the best source of flavanols (epicatechin, catechin, proanthocyanidin) known for their significant antioxidant potential (Bony *et al*. 2010) [42]. Several studies have shown the benefit of adding cocoa powder on the physicochemical and microbiological quality of milk, but few studies have looked at its antioxidant potential. Our interest in this ingredient stems from these findings and the present work is to fill the gap in this information. The objective of our work is to study the influence of the addition of cocoa powder on the quality of milk and its antioxidant potential, and this by analyzing the chemical composition and the antioxidant power of cocoa powder and the two types of UHT milks (chocolate and plain) and monitoring their stability during storage (30°C/120 days and 55°C/30 days).

Material and methods

Origin of samples

The semi-skimmed UHT milks (chocolate and plain) are provided from the *Sarl Ramy milk Company* (Company located in the industrial zone of El Harrach (region located in the North of Algeria). The cocoa powder analyzed is imported from Côte ivory.

Physicochemical analyzes

The pH is the potential difference between two electrodes immersed in the product being measured (AFNOR 2009) [2]. The titratable acidity is expressed in degree dornic (0.1 g of lactic acid/l) compared to lactic acid (AFNOR 1980, JORA 2015) [5, 29]. The total dry extract (TDE) is a drying by evaporation of a certain volume of the milk followed by weighing of the residue (AFNOR 1970) [3]. The density is determined at a temperature of 20°C, using a thermo-lactodensimeter carried out with a graduated rod (JORA 2018) [30]. The Brix degree was read directly on the refractometer scale at the intersection of the boundary between the light and dark fringes (AOAC 2002) [7].

Stability test

Stability test of milk to alcohol

This test minimizes the risk of the milk destabilizing and settling in prepackage after UHT treatment (Metro *et al*. 1979) [36]. It involves by mixing one volume of milk and one volume of ethanol in a test tube. We turn twice without stirring. If the mixture flows without leaving traces along

the sides with no precipitate or lumps for at least one minute, the milk is said to be normal. Otherwise, the stability of the milk is doubtful (Metro *et al*. 1979, Guiraud 1998) [36, 26].

Milk heat stability test or boiling test

Boiling stability is the ability of milk to undergo heat treatment without coagulation or flocculation. Milk is boiled for 10 minutes and checked for flocculation or not (Metro *et al*. 1979) [36].

Chemical composition analyzes

Defecation of samples

Milk is loaded with many substances (carbohydrates, fats and lipoids, amino acids, organic acids, mineral salts, reducing bodies which are not carbohydrates, etc.). These substances may interfere with the dosage of sugars. They must therefore be eliminated beforehand; this is the purpose of defecation. Defecation of the samples was carried out according to the method described by Carrez (1909) [16].

Total sugar content

The total sugar content was determined according to the method of Dubois *et al*. (1956) [21]. In the presence of sulfuric acid and heating, carbohydrates are dehydrated into furfural derivatives which combine easily with phenol and give further a pink-salmon color. The sugar concentrations are determined by referring to the standard glucose curve (10 to 80µg/ml) and the results are expressed in mg Glucose equivalent/100 ml of milk.

Protein content

The proteins were assayed according to the method of Bradford (1976) [14] which is based on a colorimetric assay based on the change in the color of Coomassie blue after complexation with the aromatic amino acids (tryptophan, tryosine and phenylalanine) and the residues hydrophobic amino acids present in the proteins. A calibration curve is taken from a standard solution of BSA (10 to 90µg/ml).

Ash content

The determination of ash content is based on the destruction of all organic matter under the effect of high temperature (500 ± 25°C) (AFNOR 1987).

Determination of fat (Gerber method)

The milk fat is separated in the butyrometer by centrifugation after attacking the elements of the milk with sulfuric acid except the fat. The separation of the latter is favored by the addition of iso-amyl alcohol (AFNOR 1993) [1]. Regarding the cocoa powder, the extraction of the fat was carried out by organic solvent (Hexane) with a Soxhlet type apparatus, following the method as described by ISO 659 (1998) [28].

Ascorbic acid content

Vitamin C or ascorbic acid was extracted in the presence of a solution of Meta phosphoric acid/acetic acid and assayed with 2, 6-dichlorophenol indophenol calibrated by vitamin C of known concentration (Poncraz *et al*. 1971).

Phenolic compounds in cocoa powder

The phenolic compounds were extracted three times in a mixture of 10g of cocoa powder and 100ml of acetone/water (70% v/v) and methanol / water (80% v/v) (50%/50%) solution, with magnetic stirring at room temperature for 3h, then centrifuged at 2000 rpm for 20 min at 4°C. The supernatants were mixed; the final extract is filtered with a filter syringe with a porosity of 0.45 µm and then concentrated using a rotary steamer at 40°C. Total polyphenols are quantified according to Meyers *et al*. (2003) [37], the condensed tannins are determined by the vanillin method described by Ba *et al*. (2010) [9]. The flavonoids are determined according to Bahorun *et al*. (1996) [10] by direct dosing with aluminum chloride.

Energetic value

The energy value was determined based on the carbohydrate, protein and lipid contents, taking into account the ATWATER coefficients (4kcal/g for sugars and proteins, and 9Kcal/g for lipids) (AFNOR 1987).

Antioxidant activity

In this study the antioxidant activity was evaluated using the scavenging activity of the free radical 1,1-diphenyl-1-2-picrylhydrazyl (DPPH) (Brand-Williams *et al*. 1995) [15] and 2,2'-Azino-bis (3-ethylbenzothiazoline-6-sulphonic) acid (ABTS) (Kelebek and Selli 2014) [32].

Statistic study

The statistical analysis of the results is carried out using the STATISTICA 5.5 software and the degree of significance is taken at the probability $p \leq 0.05$. We performed two-way analysis of variance for the storage stability results and one-way analysis of variance for the remainder of the results followed by a Tukey HSD Test. All data represent the mean of the three tests ± standard deviation.

Results and discussion

Chemical composition of cocoa powder

The results of table 1 show a neutral pH and an estimated moisture content of 4% for the analyzed cocoa powder. The physico-chemistry of cocoa is very complex. Indeed, depending on the varieties and the treatments that the cocoa beans undergo, the results change. Knapp (1920) [33] recorded a moisture content of 9.3% for cocoa beans while Hurst (2015) [27] found a value of 3.2% for cocoa beans after fermentation and drying. According to Gbogbri (2019) [24], the water content in fermented beans should be around 5% (10% and more being considered a sign of poor fermentation.

Table 1: Physicochemical parameters, chemical composition and energy value of cocoa powder

Physicochemical parameters	Values
pH	7.20±0.55
Moisture (%)	4.01±0.39
Chemical composition	
Protein (g BSA E /100g)	19.46±1.02
Total sugars (g Glu E /100g)	10.91±0.97
Fat (g/100g)	13.94±0.55
Ashes (g/100 g)	4.93±0.01
Vitamin C (g AAE/100g)	Nd
Total polyphenols (g GAE/100g)	5.52±0.53
Flavonoids (g QE/100g)	2.03±0.02
Condensed tannins (g CE/100g)	3.14±0.11
Energetic value (Kcal/100g)	
246.94±12.91	

AAE: *g Ascorbic Acid Equivalent, BSA E: Bovine Serum Albumin Equivalent, CE: Catechin Equivalent, Glu E: Glucose Equivalent,*

QE: Quercetin Equivalent, Nd: Not determined.

The neutral pH of the powder is due to the alkalization of the cocoa beans before roasting in order to make the cocoa more soluble in water, modify its color (increase in intensity) and lighten its aroma (disappearance of the components acids) (Daverio 2005) [19]. The cocoa powder tested is rich in protein (19.46%) and contains approximately 14% of fat, 11% of carbohydrate and 5% of ash. Our results are close to those found by Daverio (2005) [19] (12 to 18% for proteins, 13 to 15% for carbohydrates and 2 to 5% for ash). In addition, our result for lipids is consistent with the legal denomination of cocoa powder. According to the decree of 19-12-1910 replaced by the decree of 16-11-1951, the name of cocoa powder is reserved for the product of the spraying, after or without degreasing, of the cocoa mass, on the condition that the cocoa powder or cocoa powder obtained contain at least 18% cocoa butter, calculated on the dry matter. Our obtained result for total polyphenols exceeds that of Nogbou *et al*. (2015) [38] (2.13 to 4.4g/100g). This difference in results can be explained by the influence of some parameters such as pedoclimatic conditions, geographical distribution, fruit ripening stage, cultivar, genetic factors, method and conditions of extraction and quantification, … etc. (Levizou *et al*. 2004) [34]. When compared with bibliographic data, it can be clearly seen that cocoa powder stands out among foods as being the best source of flavonoids (2.03g EQ/100g). It contains twenty to one hundred times more than green apple (0.11g per 100g) and red wine (0.022g/100g), respectively. According to Bony *et al*. (2010), cocoa powder is considered to be the best source of flavanols (epicatechin, catechin, proanthocyanidin) known for their significant antioxidant potential. From the results in table 1, it can be also shown that cocoa powder contains more condensed tannins than flavonoids. According to Gbogbri (2019) [24], the bitter taste and acidic aromas of cocoa powder are due to very high tannins contents. The latter condense during the fermentation of the cocoa beans, giving the characteristic brown color of cocoa. The results of table 1 show that the overall energy value of our cocoa powder is estimated at 246.94Kcal / 100g. This value is close to that found by Vijayanand and Kulkarni (2012) [16] for date molasses (245Kcal/100g) which is rich in certain nutrients. The latter is a good source of rapid energy and serves as a source of calories with around 78% of carbohydrates, 2 to 3% of proteins and 1% of fats (Ardali and Akbarian 2014) [8].

Physicochemical parameters and chemical composition of UHT milks

The comparative study of UHT milks (Table 2) shows that the two types of milks do not differ significantly (P>0.05) in terms of acidity and ash content. Chocolate UHT milk is found to be significantly (P≤0.05) denser and richer in sugar, fat, TDE (total dry extract) and soluble solids than plain UHT milk. On the other hand, it seems to be significantly (P≤0.05) poorer in proteins. The high sugar, fat, TDE, soluble solid and density of UHT chocolate milk can be explained by the difference in composition of the two milks (the addition of cocoa and sugar, especially for chocolate milk). According to JORA, No. 69, chocolate milk is formulated with semi-skimmed milk (1 to 1.5 MG), cocoa (1.5 to 2%), sucrose (5 to 6%) and stabilizers: alginate, pectin and starch. The addition of these ingredients increases the total sugars and fat content, which promotes density, TDE and soluble solid. According to Goursoud (1985), the density of milk is related to its dry matter content. The increase in density means that the milk is enriched in dry matter; a decrease in the latter reflects enrichment in fat and depletion in dry matter. The high density of chocolate UHT milk is also supported by the addition of stabilizers. According to table 2, the protein content of chocolate UHT milk represents 89.5% of the protein content of plain UHT milk. This difference in content can be explained according to Dupas (2005) [22] and Porter (2006) [41], by the engagement of proteins in bonds with the phenolic compounds present in the cocoa powder which opposes their availability and hinders their quantification.

Table 2: Physicochemical parameters and chemical composition of UHT chocolate and plain milks

	Chocolate milk		**Plain milk**	
Physico Chemical Parameters	Results	Internal standard *Sarl Ramy milk Company*	Results	Internal standard *Sarl Ramy milk Company*
pH at 20°C	6.68±0.01^a	6.6-6.8	6.68±0.02^a	6.5 à 6.7
Acidity (°D)	14.01±0.00^a	14-16	14.00±0.00^a	14 -18
TDE (%)	18.62±0.02^a	18.6±0.1	10.58±0.01^b	10.5 à 10.7
Brix (%)	18.02±0.58^a	18±0.1	10.07±0.19^b	10±0.1
Density	1.07±0.001^a	1.068-1.071	1.03±0.00^b	1.030-1.033
Chemical composition	Results	Internal standard *Sarl Ramy milk Company*	Results	Internal standard *Sarl Ramy milk Company*
Protein (mg BSA E/ml)	45.35±0.58^b	-	49.15±0.58^a	-
Total sugars (mg Glu E/ml)	100.75±0.27^a	-	33.91±0.15^b	-
Fat (g/100ml)	1.73±0.03^a	1.5-2	1.52±0.03^b	1.5- 2
Ashes (g/100 g)	0.74±0.02^a	0.8±0.1	0.73±0.02^a	0.8±0.1
Vitamin C (g AAE/l)	7.46±0.02	-	Nd	
Total polyphenols (mg GAE/l)	150±0.46	-	Nd	
Energetic value (Kcal/100ml)	73.74±0.20^a		46.90±0.17^b	

AA E: *Ascorbic Acid Equivalent, BSA E: Bovine Serum Albumin Equivalent D: Degree Dornic, GA E: Gallic acid Equivalent, Glu E: Glucose Equivalent, Nd: Not determined. Values with the same letters on the same row did not differ significantly (p> 0.05).The results are listed in descending order: a> b.*

The total polyphenol and vitamin C contents of our chocolate milk are estimated at 150 mg GAE/l and 7.46g AAE/l, respectively (Table 2). These values far exceed those of some fruit juices such as orange juice and grapefruit juice (48 to 109mgGAE/100ml and 50mg AAE/100ml, 54mg GAE/100ml and 38mgAAE/100ml, respectively) (Favier 1995, Rapisarda and al. 1999) [43]. In view of this comparison, chocolate milk would therefore be an interesting source of vitamin C and phenolic compounds.

The results of Table 2 show that the overall energy values of chocolate and plain milks are estimated respectively at 73.74Kcal/100ml and at 46.90Kcal/100ml. These values are close to those found by European Union regulation No. 1169/2011 for semi-skimmed chocolate and plain UHT milks (68Kcal/100g and 46Kcal/100g, respectively)

Study of the antioxidant potential of cocoa powder and UHT milks

Antioxidant activity was evaluated using the free radical scavenging methods 1, 1-diphenyl-1-2-picrylhydrazyl (DPPH•) and 2, 2'azinol-bis-(3-ethylbenzothiazoline acid-6-sulfonic) ($ABTS^{+\bullet}$). Table 3 gives the average values of the antioxidant activities of the samples studied. The results show that the antioxidant potential of the two free radicals ($DPPH^{\bullet}$ and $ABTS^{+\bullet}$) of chocolate milk is significantly ($p \leq 0.05$) higher than that of plain milk (85.5% and 75.9% against 3%, 18% and 1.05%) but significantly lower ($p \leq 0.05$) than vitamin C (1mg/ml) (93.26% and 85.02%, respectively) and cocoa powder (1mg / ml) (96.02% and 89%, respectively). The anti-free radical activity of chocolate milk is 96 to 98.6% higher than that of plain milk, 8.3% to 10.73% and 10.95% to 14.72% lower than that of vitamin C and of cocoa powder tested at the concentration of 1 mg/ml.

Our results show that plain milk has discreet antioxidant activity. Cloetens *et al*. (2013) [17] also recorded a discreet antioxidant capacity for commercial UHT milks (13µmol Trolox/ml). Dairy products contain antioxidant compounds in varying proportions depending on the matrices and the technological processes applied. These compounds include the protein fraction (more specifically casein), antioxidant enzymes (superoxide dismutase, catalase and glutathione peroxidase), lactoferrin, vitamins E, A and D3, conjugated linoleic acid, coenzyme Q, equol (Tsen *et al*. 2014) [44], uric acid and certain carotenoids (Lindmark-Mansson and Akesson 2000) [35]. The high antioxidant activity of chocolate milk may be due to the presence of vitamin C and the richness of cocoa powder in polyphenols known for their high antioxidant activity. Porter (2006) [41] report that cocoa powder contains 2% polyphenols. The polyphenols in cocoa are mainly flavanols and a few rare hydroxycinnamic derivatives. Flavanols clearly dominate; they alone represent 90% of the polyphenols in cocoa. They are essentially procyanidins: the monomer is (-) - epicatechin, the dimers are procyanidin B_2 and procyanidin B_5, and the trimer is procyanidin C_1. According to Bardoulat (2005) [11], The antioxidant activity demonstrated for these compounds may also be the result of a synergy with equally powerful but less abundant antioxidants present in cocoa such as vitamins E and C. The very pronounced antioxidant activity of vitamin C compared to chocolate milk which contains vitamin C, milk proteins with antioxidant power and phenolic compounds from cocoa powder can be explained by the formation of bonds between polyphenols and milk proteins, which reduces the antioxidant power of the mixture (Dupas 2005) [22].

Table 3: The scavenger powers of DPPH and ABTS in UHT milks (chocolate and plain), vitamin C and cocoa powder at a concentration of 1 mg/ml.

	Vitamin C (1mg/ml)	Cocoa powder (1mg/ml)	Chocolate milk	Plain milk
DPPH scavenger power (%)	93.26 ± 0.15^a	96.02 ± 0.07^a	85.5 ± 0.11^a	3.18 ± 0.07^a
ABTS scavenger power (%)	85.02 ± 0.22^b	89 ± 0.05^b	75.9 ± 0.05^b	1.05 ± 0.11^b

***ABTS:** 2, 2'-azinol-bis- (3-ethylbenzothiazoline acid-6-sulfonic), DPPH: 1, 1-diphenyl 1-2-picrylhydrazyl, Values with the same letter, on the same line, do not show significant differences (P> 0.05). The results are listed in descending order: a> b.*

Stability study of UHT milks

Chocolate and plain UHT sterilized milks, stored at two temperatures and for two different times (30°C for 120 days and 55°C for 30 days), are compared to their counterparts dated one day of storage at room temperature. From the obtained results (Table 4), there is a stability of the physicochemical parameters (TDE, Brix, density and acidity) after the storage period for the two types of milk. There is also no variation in the polyphenols and fat content. On the other hand, there is an effect of storage time and temperature on other chemical compounds in UHT milks. There is a significant decrease ($p \leq 0.05$) in ash, protein and vitamin C levels after storage at 30°C for 120 days while for the sugar level, the decrease is proportional to the increase in temperature and it is more significant for plain milk. We note that despite the instability of the levels of these compounds, UHT milks keep their compliance with the standards required by the *Ramy Milk Company*. The content of vitamin C is influenced by heat and light. According to Allen and Joseph (1985) [6], milk contains 8 to 9 mg of oxygen per liter. In the presence of this dissolved gas, losses of vitamin C are even greater during storage. In accordance with Bosset *et al*. (1992) [13], the loss of vitamin C is in the order of 10% per 24 hours in the dark. Datta and Deeth (2003) [18] report that during UHT processing of milk and during subsequent storage at room temperature, several changes occur which affect the quality and shelf life of the final product. The changes include denaturation of whey protein, protein-protein and lactose-protein interactions, and isomerization of lactose to lactulose. This explains the discrete loss of proteins and sugars in the milk studied during the two storage conditions. According to Pougheon and Goursaud (2001) [42], the decrease in the ash content during storage at 55 ° C can be explained by a decrease in soluble calcium which passes into the micellar phase and becomes insolubilized with the increase in temperature and conversely, lowering the temperature leads to partial solubilization of the micellar calcium. The results also show that the antioxidant potential is stable for chocolate milk, whereas it significantly decreases in the same way ($p \leq 0.05$) during the two storages for plain milk. The stability of the antioxidant potential of chocolate milk compared to plain milk can be justified by its richness in vitamin C and phenolic compounds which reinforces and maintains the

antioxidant status of products to the detriment of dairy proteins with antioxidant potential denatured or polymerized during heat treatment during storage. The results for heat and alcohol tests are negative; the two types of milk show neither precipitation, nor flocculation, nor coagulation, which reveals that the UHT milk analyzed is heat stable.

Table 4: Physicochemical parameters, chemical composition and antioxidant potential of UHT chocolate milk and plain after storage

Chocolate milk	**Plain milk**					
Physicochemical parameters						
parameters	1st day	30°C/120 days	55°C/30days	1st day	30°C/120 days	55°C/30days
pH at 20°C	6.72±0.02^{a}	6.79±0.07^{a}	6.79±0.07^{a}	6.68±0.02^{b}	6.69±0.03^{b}	6.68±0.01^{b}
Acidity (°D)	14.00±0.00^{a}	13.66±0.58^{a}	13.67±0.58^{a}	14.00±0.00^{a}	14.00±0.00^{a}	13.67±0.58^{a}
TDE (%)	18.82±0.04^{a}	18.74±0.11^{a}	18.71±0.01^{a}	10.52±0.04^{b}	10.54±0.02^{b}	10.51±0.11^{b}
Brix (%)	18.33±0.58^{a}	18.33±0.58^{a}	18.33±0.58^{a}	10.17±0.29^{b}	10.17 ±0.29^{b}	10.17 ±0.29^{b}
Density	1.070±0.001^{a}	1.07±0.00^{a}	1.07±0.00^{ab}	1.03±0.00^{c}	1.03±0.00^{c}	1.03±0.00^{c}
Alcohol stability	Negative			Negative		
Boiling stability	Negative			Negative		
Chemical composition						
Compounds	1st day	30°C/120 days	55°C/30days	1st day	30°C/120 days	55°C/30days
Proteins(mg BSAE/ml)	45.25±0.58^{d}	43.55±0.23^{e}	44.82±0.42^{d}	49.15±0.58^{a}	46.87±0.60^{c}	48.49±0.09^{ab}
Total sugars (mgGluE /ml)	100.75±0.27^{a}	100.4±0.18^{ab}	94.37±0.66^{c}	33.91±0.15^{d}	30.92±0.13^{e}	27.22±0.19^{f}
Fat (g/L)	1.73±0.03^{a}	1.70±0.00^{a}	1.70±0.00^{a}	1.52±0.03^{b}	1.50±0.00^{b}	1.50±0.00^{b}
Ashes (g/100 g)	0.74±0.02^{a}	0.73±0.02^{c}	0.66±0.00^{e}	0.73±0.02^{a}	0.71±0.00^{b}	0.65±0.00^{d}
Vitamin C (g AAE/L)	7.46±0.02^{a}	7.31±0.01^{b}	7.45±0.01^{a}	Nd		
Total polyphenols	150±0.46^{a}	149.57±0.11^{a}	150.17± 0.01^{a}	Nd		
Antioxidant activity (DPPH)	85.5±2.23^{a}	83.07±0.11^{ab}	84.07±1.02^{ab}	3.18±0.32^{c}	2.80±0.05^{d}	2.73±0.01^{e}

AAE: *Ascorbic Acid Equivalent, BSA E: Bovine Serum Albumin Equivalent D: Degree Dornic, DPPH: 1, 1-diphenyl 1-2-picrylhydrazyl, GA E: Gallic acid Equivalent, Glu E: Glucose Equivalent, Nd: Not determined. Values with the same letters on the same row did not differ significantly (p> 0.05). The results are listed in descending order: a> b> c> d> e>f.*

Conclusion

Our work aims to study the influence of the addition of cocoa powder on the quality of milk, its antioxidant potential and its stability during storage (30°C/150 days, and 55°C/30 days). The results of the analysis of the chemical composition of the cocoa powder tested show that it is rich in protein (19.46%) and contains approximately 14% fat, 11% carbohydrate and 5% ash. Colorimetric assays show that it is very rich in phenolic compounds; it contains more condensed tannins than flavonoids and shows a powerful antioxidant potential with regard to stable radials DPPH and ABTS. The results also show that the two types of milk do not differ significantly (P>0.05) in terms of acidity and ash content. Chocolate UHT milk is found to be significantly ($p \leq 0.05$) denser and richer in sugar, fat, TSE and soluble solids than plain UHT milk. On the other hand, it seems to be significantly ($P \leq 0.05$) poorer in proteins. The experimental data also show that the antioxidant potential of chocolate milk is significantly ($p \leq 0.05$) higher than that of plain milk, the increase is from 96% to 98.6% This may be due to the presence of vitamin C and the richness of cocoa powder in polyphenols known for their high antioxidant activity. The stability study does not show any variation in the physicochemical parameters (TDE, Brix, density and acidity) and the fat contents after storage for 120 days at 30 ° C and 30 days at 55 ° C for the two types of milks. On the other hand, there is an effect of storage time and temperature on other chemical compounds in milk. There is a significant decrease ($p \leq 0.05$) in ash, protein and vitamin C levels after storage at 30 ° C for 120 days while for the sugar level, the decrease is proportional to the increase in temperature and it is more significant for plain milk. We note that despite the instability of the levels of these compounds, UHT milks keep their compliance with the standards required by the Ramy Milk Company. The results also show that the antioxidant potential is stable for chocolate milk, whereas it significantly decreases in the same way ($p \leq 0.05$) during the two storages for plain milk. The stability of the antioxidant potential of chocolate milk compared to plain milk can be justified by its richness in vitamin C and phenolic compounds which strengthen and maintain the antioxidant status of products to the detriment of milk proteins, with antioxidant potential, denatured or polymerized during the production heat treatment during storage.

References

1. AFNOR. Association Française de Normalisation. Recueil des normes de contrôle de qualité des produits alimentaires: lait et produits laitiers : analyses physicochimiques 4eme Ed la défense. Paris, 1993, 581.
2. AFNOR. Association Française de Normalisation. Lait et produits laitiers : détermination du pH (indice de classement) (NF V04-035), 2009.
3. AFNOR. Association Française de Normalisation. Détermination de la matière sèche du lait (indice de classement) (NF V04-207), 1970.
4. AFNOR. Association Française de Normalisation. Lait : détermination de l'acidité titrable (méthodes pratiques).1er Ed (ISO 6092), 1980.
5. AFNOR. Association Française de Normalisation. Recueil des normes des méthodes générales d'analyses des produits agroalimentaires. Bulletin norme VF 09-012, 3eme Ed. Paris, 1980.
6. Allen JC, Joseph G. Deterioration of pasteurized milk on storage. Journal of Dairy Research,1985:52:469-487
7. AOAC. Official Methods of Analysis. 17th Ed. Gaithersburg, USA, 2002, 480.
8. Ardali FR, Akbarian M. The Influence of Date Syrup on Color Texture and Sensory Properties of Gaz.

Bulletin of Environment Pharmacology and Life Sciences,2014:3(2):159-163.
9. Ba K, Tine E, Destain J, Cisse N, Onart P. Etude comparative des composés phénoliques du pouvoir anti-oxydant de différentes variétés de sorgho sénégalais et des enzymes amylolytiques de leur malt. Biotechnology Agronomy and Society and Environment,2010:14:131-139.
10. Bahorun T, Gressier B, Trotin F, Brunet C, Dine T, Luyckx M *et al*. Oxygen species scavenging activity of phenolic extracts from hawthorn fresh plant organs and pharmaceutical preparations. Arzneimittel Forschung,1996:46:1086-1108
11. Bardoulat M. Le chocolat du plaisir à la santé : de la fève au chocolat tous les bienfaits du cacao. Ed Alpen, France, 2005, 39.
12. Bony FN, Gbassi GK, Yolou FS, Atheba PG, Trokourey A, Amin CN *et al*. Extraction et identification de polyphénols de la poudre de cacao. Journal of Pharmaceutical and Biological Sciences,2010:11(1):38-46.
13. Bosset JO, Eberhard P, Butikofer U, Sieber R. Evaluation de quelques critères d'altération du lait entier soumis à divers traitements thermiques et mécaniques ainsi qu'à diverses durées d'exposition à la lumiere : Partie I: Etude de la vitamine C. FAM-Information,1992:236:1-25.
14. Bradford MM. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry,1976:72:248-254.
15. Brand-Williams W, Cuvelier ME, Berset C. Use of free radical method to evaluate antioxidant activity. LWT – Journal of Food Science and Technology,1995:28(1):25-30.
16. Carrez MC. Defecation du lait pour le dosage du lactose par les liqueurs cupriques. Phamaceutical Chemistry Journal,1909:30:36-37.
17. Cloetens L, Panee J, Akesson B. The antioxidant capacity of milk--the application of different methods *in vitro* and *in vivo*. Cellular and Molecular Biology,2013:59(1):43-57.
18. Datta N, Deeth HC. Diagnosing the cause of proteolysis in UHT milk Lebensmittel Wissenschaft and technology. Food Science and Technology,2003:36(2):173-182.
19. Daverio S. Le chocolat dans tous ses états. Thèse de doctorat en pharmacie. Université Henri poincare, Nancy I, 2005, 163.
20. Debry G. Lait nutrition et santé. Ed Lavoisier Tec et Doc, Paris, 2001, 566.
21. Dubois M. Colorimetric method for determination of sugars and related substances. Analytical Chemistry,1956:28(3):350-356.
22. Dupas C. Influence des protéines laitières sur le pouvoir antioxydant et la biodisponibilité des polyphénols du café. Thèse de doctorat en Sciences Alimentaires. Ecole Nationale supérieure des industries agroalimentaires Agricoles *et al*imentaires « ENSIA » (Agro Paris Tech), France, 2005.
23. Favier A. Stress oxydant et pathologies humaines. Annales pharmaceutiques françaises,2006:64(6):390-396.
24. Gbogbri GF. Impact de la fermentation sur les propriétés anti-oxydantes anti-inflammatoires et immuno-modulatrices du cacao. Thèse de Doctorat en Biochimie et physico-chimie alimentaires, Université Montpellier, France, 2019.
25. Goursaud J. Composition et propriétés physico-chimiques propriétés In : Laits et produits laitiers vache brebis chèvre. Tome 1 Ed Lavoisier Tec et Doc, Paris, 1985.
26. Guiraud JP. Microbiologie alimentaire. Ed DUNO, Paris, 1989, 696.
27. Hurst JW. Chocolate and Health: Chemistry Nutrition and Therapy. In: Wilson K & Jeffrey Hurst W (Ed) Royal Society of Chemistry. 1st Ed, 2015, 1-260.
28. ISO International Organization for Standardization. Oléagineux- Détermination de la teneur en huile (méthode de référence), 1998.
29. JORA. Journal Officiel de la République Algérienne N° 58 Arrêté du 18 octobre rendant obligatoire la méthode de détermination de l'acidité titrable dans le lait sec, 2015.
30. JORA. Journal Officiel de la République Algérienne N° 38 Arrêté du 24 Juin rendant obligatoire la méthode de détermination de la masse volumique du lait et de certains produits laitiers en poudre, 2018.
31. JORA. Journal Officiel de la République Algérienne N°69 Arrêté interministériel du 18 Août relatif aux spécifications et à la présentation de certains laits de consommation, 1993.
32. Kelebek H, Selli S. Identification of phenolic compositions and the antioxidant capacity of mandarin juices and wines. Journal of Food Science and Technology,2014:51(6):1094-1101.
33. Knapp AW. Cocoa and chocolate: Thier history from plantation to consumer. London, 1920, 191.
34. Levizou E, Karageorgou P, Petropoulou Y, Grammati kopoulos G, Manetas Y. Induction of ageotropic response in lettuce radicle growth by epicuticular flavonoid aglycons of Dittrichia viscose. Biologia Plantarum,2004:48:305-307.
35. Lindmark-Månsson H, Akesson B. Antioxidative factors in milk British. Journal of Nutrition,2000:84(1):103-110.
36. Metro F, Desmazeaud MJ, Cerf OF. Facteurs influençant sur la validité de l'épreuve à l'alcool utilisé pour la stérilisation des laits stables à la chaleur Le lait. Ed INRA, France,1979:59:431-448.
37. Meyers K, JWatkins CB, Pritts MP, Liu RH. Anti oxydant and antiproliferative activities of strawberries. Journal of Agricultural and Food Chemistry ,2003:51(23):6887-6892.
38. Nogbou LI, Akmel DC, Brou K & Assidjo EN. Etude du séchage microonde par intermittence sur la qualité physicochimique des fèves de cacao Arsène., Revue Ivoirienne des Sciences et Technologie,2015:26:18-35.
39. Pongracz G, Weiser H, Matzinger D. Tocopherols-Antioxydant Fat Science Technology,1971:(97):90-104.
40. Pongracz, Weiser H, Matzinger D. Tocopherols: Antioxydant. Fat Science Technology,1971:(97):90-104
41. Porter LL. Benefits of Cacao polyphenols. Manufacturing confectioner, 2006, 49.
42. Pougheon S, Goursaud J. Le lait et ses constituants: caractéristiques physicochimiques. In : DEBRYG Lait nutrition et santé. Ed Lavoisier Tec et Doc, Paris, 2001, 342

43. Rapisarda PA, Tomaino CRL, Bonina F, De Pasquale A, Saija A. Antioxidant effectiveness as influenced by phenolic content of fresh orange juices. Journal of Agricultural and Food Chemistry,1999:47:4718-4723.
44. Tsen SY, Siew J, Lau EKL, Farzana ABR. Cow's milk as a dietary source of equol and phenolic antioxidants: differential distribution in the milk aqueous and lipid fractions. Dairy Science Technology,2014:94:625-32.
45. Vigniola CL. Science et technologie du lait : Transformation du lait Presses inter polytechnique. Montréal, Quèbec, 2002, 600.
46. Vijayanand P, Kulkarni SG. Processing of Dates into Value-Added Products In: Manickavasagan A Mohamed Essa M & Sukumar E Production Processing. Food and Medicinal Values CRC, Press New York, 2012, 255-262
47. Yakhlef H, Madani T, Ghozlane F, Bir A. Rôle du matériel animal et de l'environnement dans l'orientation des systèmes d'élevage bovins en Algérie. 8ème Journées des Science Vétérinaire 18 et 19 Avril Ecole Nationale Supérieure Vétérinaire d'Alger, 2010. (https://wwwensvdz/wp-content/uploads/2016/01/8JSV_progwebpdf).