Résumé

La grenade a attiré un grand intérêt des scientifiques du monde entier, grâce à la fois sa composition chimique et sa valeur sensorielle. Le but de ce présent travail est d'étudier la stabilité thermique des anthocyanes et les paramètres physico-chimiques des trois formulations de jus de grenade (*Punica granatum* L.), Jus Contrôle Non Fortifié (NFCJ), Jus Fortifié avec l'Acid Ascorbique à 100 ppm (AAFJ-100) et Jus Fortifié avec l'Acide Ascorbique à 200 ppm (AAFJ-200) obtenu à partir des arilles isolées, qui sont traités thermiquement à différentes couples températures-temps (60, 70, 80 et 90 °C de 5 min à 180 min). Les résultats ont révélé un effet négatif de l'acide ascorbique sur la préservation des anthocyanes à concentration élevée (200 ppm) et un effet destructeur de la température à des degrés et des temps d'exposition plus élevés, les paramètres physico-chimiques sont légèrement affectés par la température et la fortification avec ascorbique acide.

Des yaourts étuvés ont été préparé en fortifiant le lait avec du jus de grenade frais, jus de grenade pasteurisés à 60 °C/15 min et jus de grenade commercialisé, les produits finaux ont été soumis à des analyses physicochimiques, microbiologiques et sensorielles révélant de meilleur propriétés antioxydants et organoleptiques.

Mots clés : jus de grenade, acide ascorbique, pasteurisation, yaourt.

Abstract

Pomegranate fruits have attracted huge interest among scientists worldwide, due to both their chemical composition and sensory value. The aim of the present work is to study the thermal stability of anthocyanins and physicochemical parameters of three pomegranate juices (*Punica granatum* L.), Non-Fortified Control juice (NFCJ), Ascorbic Acid Fortified Juice at 100 ppm (AAFJ-100) and Ascorbic Acid Fortified juice at 200 ppm (AAFJ-200) obtained from isolated arils upon heating at 60, 70, 80 and 90 °C from 5 min to 180 min. The results revealed a negative effect of Ascorbic Acid fortification on anthocyanin preservation at high concentration (200 ppm) and a destructive effect of temperature at higher degrees and exposure time, the physicochemical parameters are slightly affected by both temperature and acid ascorbic fortification.

Steamed yoghurts were prepared by fortifying milk with fresh pomegranate juice, pasteurized pomegranate juice at 60 °C/15 min and commercialized pomegranate juice, the final products were subjected to physicochemical, microbiological and sensory analysis revealing better antioxidant and organoleptic properties.

Keywords: pomegranate juice, ascorbic acid, pasteurization, yoghurt.

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Département des Sciences Alimentaires
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Spécialité : Science alimentaire
Option : Industrie Laitière

Réf :.....

Mémoire de Fin de Cycle
En vue de l'obtention du diplôme

MASTER

Thème

Etude de la dégradation des anthocyanes du jus de grenade lors d'un procédé de transformation thermique et élaboration d'un yaourt

Présenté par :

LANDRI Kenza & BOULAHBAL Nawel

Soutenu le : **15 Juin 2016**

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DEDICATION

For those which gave me everything without anything in return

There are no words to describe how much my parent has meant to me throughout all my life.

Mom, you have given me so much, thanks for your faith in me, and for teaching me that I should never give up. Thanks for lending me your ear on countless occasions when I needed to vent my frustrations …

Daddy, you have always been there for me with encouraging words
Thank you for your love and support. Without you, my life would fall apart.

To my sisters Sonia her husband Youcef and children, Rebha and her husband Djremy . My brothers Saber and his wife Djoher and son Nutnut, Aziz and his wife Soumia and daughter Linoucha and Noredine

To my future husband Nadjim and all his family, you are everything for
me, thanks for your patience, encouraging words and understanding ,
To my uncles and aunts , cousins and cousins
To my binomial and friend with which I have division a good moment Boulahbal Nawel and her family
To all my friends and colleagues.

Kenza

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Thank you for your love and support. Without you, my life would fall apart.

To my sisters Saida, Asma and her husband Nabil and children, Akila and her husband Abdnour and children. My brothers Rachid and his wife Amel and children , Noredine, Lamine and his wife Zina and children, Mohammed.

To my uncles and aunts , cousins and cousins
To my binomial and friend with which I have division a good momentLandri Kenza and her family

To all my friends and colleagues.

Nawel

List of abreviation

AOAC : Association of Official Agricultural Chemists

AIJN: Association of Industry of Juices and Nectar

ANOVA **:** Analysis Of Variance

ANC: Anthocyanin

TPC **:** Total Phenolic Compounds

°C: Celcus degrees

DPPH : 2,2-Diphenyl-picrylhydrazyl

TFC: Total Flavonoid Content

GAE **:** Gallic Acid Equivalent

MAE : Microwave Assisted Extraction

TDM : Total dry Matter

pH : hydrogen potential

RSA: Radical Scavenging Activity

PON1: Paraoxonase 1

LDL : Low-Density Lipoprotein

GST : Glutathion-S-Transférase

PJ : Pomegranate Juice

PJC : Pomegranate Juice Concentrate

ox-LDL: oxidized Low-Density-Lipoprotein

TG: Triglycerides

HDL-C: Highdensity Lipoprotein Cholesterol

LDL-C: Low-Density Lipoprotein Cholesterol

TC: Total Cholesterol

fig: figure

NFCL: Non-Fortified-control-juice

AAFJ-100: Acid-Ascorbic-Fortified-juice with 100mg/l

AAFJ-200: Acid-Ascorbic-Fortified-juice with 200mg/l

JCI: Juice Color Intensity

TSS: Total Soluble Solid

TA: Titritable acidity

RSA: Radical Scavenging Activity

3 BS: Biomathematics, Biochemistry, Biophysics and Scientometrics

S. aureus

S. epidermis

GAE: gallic acid equivalent

PPE: pomegranate peel extract

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Chapter I: Bibliographical Part

Experimental Part

Chapter II: Materials and methods

Chapter III: Results and discussion

Introduction

Introduction

It has been known for many centuries that a clear relationship exists between the food we eat and our health (**Rincon-Leon, 2003**). Among this food, pomegranate (*Punica granatum* L.) which is an ancient, beloved plant and fruit. The pomegranate and its usage are deeply embedded in human history, and utilization is found in many ancient human cultures as food and as a medical remedy. These findings have led to a higher awareness of the public to the benefits of the pomegranate fruit, particularly in the western world, and consequently to a prominent increase in the consumption of its fruit and juice. Despite this fact, pomegranate culture has always been restricted and generally considered as a minor crop **(Holland *et al.,* 2009**).

One of the most important quality characteristics of the pomegranate is the red pigmentation of its seeds and juice; conferred by its rich phenolic compounds especially anthocyanins.

Pomegranate juice flavour can be described by the characteristics of sour, sweet, and astringent mouth feel. These flavour characteristics are important to consider as these often determine consumer acceptance or rejection of the product (**Koppel *et al.,* 2014**). Therefore, a minimum content of pomegranate anthocyanins (ANC) should be guaranteed for the entire commercial duration of a product (**Scordino *et al.,* 2015**). However, the anthocyanins have a tendency to fade during processing and storage and consequently to give an undesirable muddy colour to the juice (**Kimball, 1999**). Otherwise, obtaining a strong, stable and fresh like colour of fruits and juices, is problematic during processing and storage (**Rein, 2005; Yu *et al.,* 2013**); since anthocyanin (ANC) stability is also affected by the intrinsic properties of the product and the process such as pH, storage temperature, chemical structure and concentration of anthocyanins present, proteins, light, oxygen, enzymes and metallic ions (**Patras *et al.,* 2010; Rein, 2005**).

During the last six decades, general interest and research activities focusing on anthocyanins have considerably increased. This increased interest is not only based on knowledge that these pigments can be used as possible alternatives to artificial food colorants and it relates to their bioactive properties (**Remini, 2015**).

Pomegranate fruit arils are also very popular due to their taste. The arils are processed to delicately flavoured juice, squash, jelly, jam, wine, etc. Due to the rich colour, sweet-sour flavour and high antioxidants content, manufacturers tend to add pomegranate to products such as jelly, ice creams, truffles and chewing gum (**Nanis *et al.*, 2014**).

Yoghurt with added antioxidants from natural sources appears to be a convenient food format to satisfy consumer interest in original yoghurt nutrients, beneficial effects of starter cultures, and health benefits of added antioxidants. For this reason, several attempts to produce yoghurts fortified with natural antioxidant-rich extracts have been undertaken, including supplementation with polyphenols-rich wine extract (**Chouchouli *et al.*, 2013**). In this context formulation of flavoured steamed yoghurt at laboratory scale was performed.

Bibliography

I-1- Overview of Pomegranate

The pomegranate tree has been known since ancient times and has traditionally been cultivated in the Near East, later spreading to the rest of Asia and the Mediterranean. It is now cultivated in all five continents: firstly, because its cultivation offers exceptional prospects, it is profitable, it can be grown in arid regions, it has less hydric requirements than other crop types and it can grow and produce in conditions which would not be possible for other more important fruit trees to do profitably; secondly, because, in the last ten years, it has been found to have properties which can prevent and possibly cure some illnesses. (**Melgarejo et Valero, 2012**).

Vernacular names

Kabyle: Remman, taremmant; **Arabic:** Rumman, shajraturrumman; **French:** Granade; **English:** Pomegranate.

- Its centre of origin is the Near East (Vavilov's Centre IV) (**Fig. 01**), spreading to different regions where it is cultivated and where it has a wide genetic diversity as a consequence of the propagation from its seeds, which are scattered by man, by birds or other animals, and germinate easily **(Sanchez-Monge, 1974).**

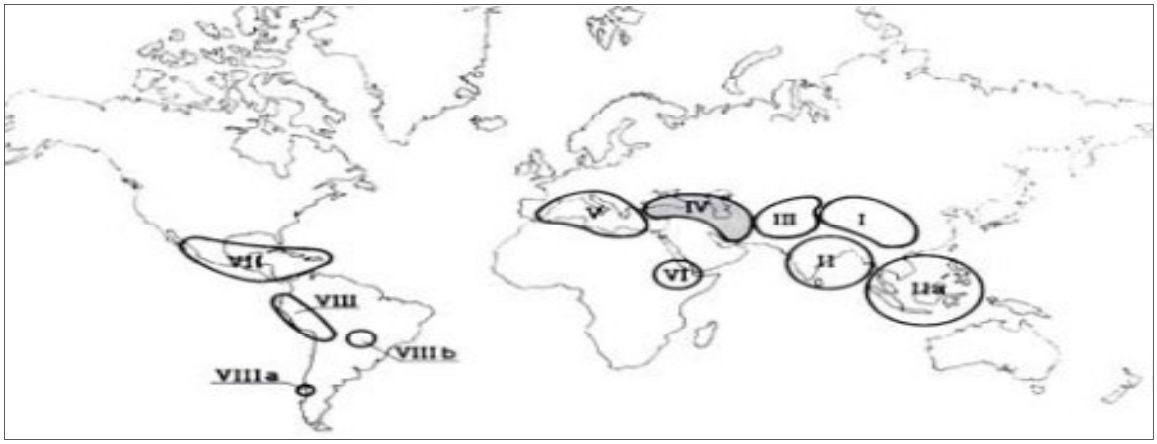


Figure 01: Centers of origin and diversity of cultivated plants, according to Vavilov (**Sanchez-Monge ,1974).**

- There are no reliable data about world cultivated area and pomegranate production; probably because it is considered a minor fruit tree. From the data provided by different researchers and associations, an estimation was made about world pomegranate production (**Fig. 02**). The data provided does not, therefore, correspond to an exact year and constitutes an estimation based on different sources. **(Melgarejo et Valero, 2012**).

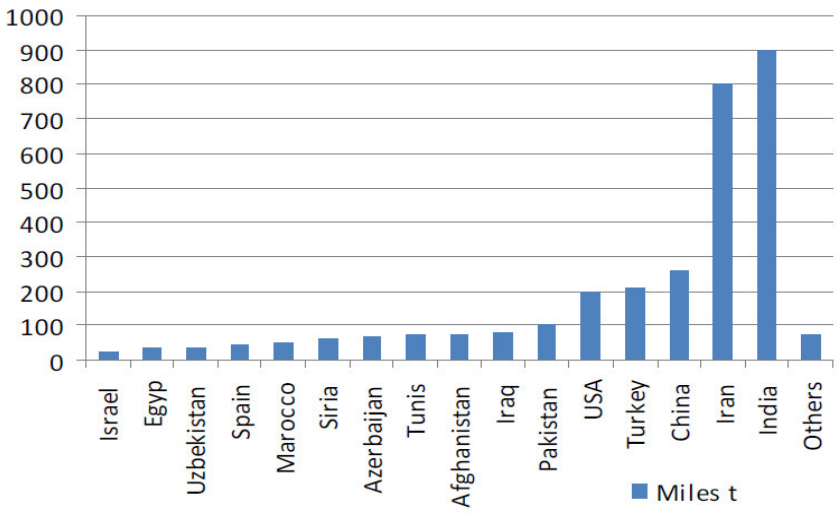


Figure 02: Estimation of world pomegranate production: 3,086 thousand tones **(Quiroz, 2009).**

I-2- Morphological description

Pomegranate (*Punica granatum* L.) is typically a shrub or a small tree with an average height of 3–5 m. Leaves are small and elliptic. The red flowers have from 5 to 7 petals and are bisexual. Fruits are orange-sized berries. They are covered by a thick rind ranging in color from yellow to red **(Holland *et al.*, 2009).** Berries are characterized by the presence of internal thin membranes, suspending and protecting seeds surrounded by juicy sarcotestas (aril). When ripe the fruits burst releasing the seeds. **(Fischer *et al.*, 2011; Holland *et al.*, 2009)**. The edible part of the fruit, the sarcotesta, accounts for 52% of entire fruit weight, including 78% juice coming from sarcotestas and 22% seeds.

Kingdom: Plantae.
Division: Magnoliophyta.
Class: Magnoliopsida.
Order: Myrtales.
Family: Punicaceae.
Genus: Punica.
Species: Granatum.

Figure 03: Taxonomic classification of *Punica granatum*.(**Nizamul *et al.*, 2015**).

I-3- Chemical composition of pomegranate

The chemical composition of the fruits differs depending on the cultivar, growing region, maturity, cultivation practice, climate, and storage circumstances **(Fadavi *et al.*, 2005**). The edible part of the pomegranate fruit (50%) consists of 40% arils and 10% seeds. Arils contain 85% water, 10% total sugars, mainly fructose and glucose, and 1.5% pectin, organic acid,

such as ascorbic acid, citric acid, and malic acid, and bioactive compounds such as phenolics and flavonoids, mainly anthocyanins. **(Viuda-Martos *et al*., 2010**).

Table 01: Constituents of pomegranate juice.

Bioactive components	Content	Reference
Anthocyanins - Cyanidin 3,5-diglucoside - Delphinidin 3-glucoside - Cyanidin 3-glucoside - Pelargonidin 3-glucoside.	6.1 - 219 mg/L 53.0 mg/L 45 – 69 mg/L 128.3 mg/L 5.9 mg/L	**(Ozgen *et al.*, 2008)** **(Gil *et al.*, 2000)** **(Miguel *et al*., 2004)** **(Gil *et al.*, 2000)**
Tannins - Gallagyl-type tannins • Punicalin (4,6-(S,S)-Gallagyl-D-glucose) • Punicalagin (2,3-(S)-hexahydroxydiphenoyl-4,6-(S,S)-gallagyl-D-glucose). - Ellagic acid derivatives.	67.9 mg /L 1500 - 1900 mg/L	**(Kamau, 2007)** **(Larrosa *et al.*, 2010)**
Phenolic acids - Gallic acid - Caffeic acid - Catechin - Ellagic acid	4.55 ± 8.55 mg/L 0.78 ± 0.79 mg/L 3.72 ± 2.29 mg/L 15.3 - 637.7 mg/L	**(Poyrazoğlu *et al.*, 2002)** **(Viladomiu *et al.*, 2013; Gil *et al.*, 2000).**
Sugars Glucose, Fructose, Sucrose, Sorbitol.	11.8 - 22 g /100g	**(Legua *et al.*, 2012; Melgarejo et al., 2000).**
Organic acids Citric acid, Malic acid, Tartaric acid, Isocitric acid	0.3 - 2.9 g /100g with citric acid predominating.	**(Melgarejo *et al.*, 2000).**
Vitamins - Ascorbic acid or vitamin C - Vitamin E (α-tocopherol).	4 - 6 mg/100g	**(*Codex Alimentarius* 2009)**
Minerals Iron, Copper, Sodium, Magnesium, Potassium, Calcium, Zinc, Manganese, Phosphorus.	Potassium, the predominant mineral, (2093- 2517 mg/L) followed by phosphorus, magnesium and calcium The amount of K, Ca and Na were highest in both juice and seeds followed by Mg, P, Zn, Fe and Cu.	**(Ekşi et Özhamamcı, 2009)** **(Al-Maiman et Ahmad, 2002)**

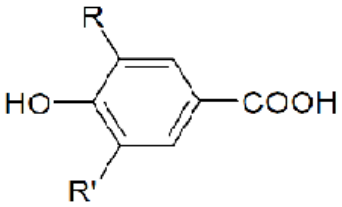
I-4- Phenolic composition

Phenolic compounds include a wide range of chemicals comprising at least one aromatic ring and one or more hydroxyl groups, in addition to other constituents (**Salunkhe, 1990**). Natural polyphenols range from simple molecules to highly polymerized compounds, the most important are: phenolic acids, anthocyanins, flavonoids and tannins. (**Hmid, 2013**).

I-4-1- Phenolic acids

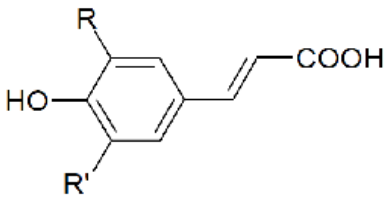
There are two main classes of phenolic acid; the derivatives of benzoic acid (C1-C6) **(Guignard, 1996)** and derivatives of cinnamic acid (C3-C6) (**Fig.04), (Malagas, 1992).** The concentration of the hydroxybenzoic acid is generally very low in edible vegetable. These derivatives are quite rare in the human diet by those against hydroxycinnamic acids which are very present **(Fleuriet *et al.*, 2005).** They are mainly present in the pomegranate by the presence of gallic acid and ellagic acid **(Amakura *et al.*, 2000).**

Benzoic acid derivatives



R = R' = H; *p*-hydroxybenzoic acid
R = OH, R' = H; protocatechuic acid
R = OCH3, R' = H; vanillic acid
R = R' = OH; gallic acid
R = R' = OCH3; syringic acid

Cinnamic acid derivatives



R = R' = H; *p*-coumaric acid
R = OH, R' = H; caffeic acid
R = OCH3, R' = H; ferulic acid
R = R' = OCH3; sinapic acid

Figure 04: Benzoic acid and cinnamic acid derivatives (**Seabra *et al.*, 2006**).

I-4-2- Flavonoids

The term flavonoids bring together a very wide range of natural compounds. Their main function seems to be the color of plants (beyond chlorophyll, carotenoids and betalains) **(Gabor, 1988).** Flavonoids have a common biosynthetic origin and they all have the same basic skeleton fifteen carbon atoms composed of two aromatic units, cycle C6 (A and B), linked by a chain C3. **(Bruneton, 1999).** The various sub groups are flavones, flavonols, flavanols, isoflavones, flavanones and anthocyanins witch are the major flavonoid of pomegranate. (**Fig .05**), (**Chira *et al.*, 2008**).

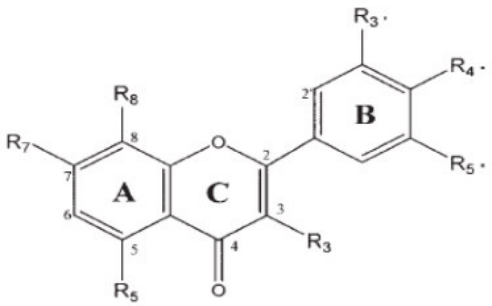
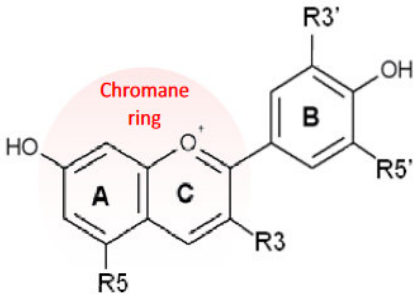


Figure 05: General structure of flavonoid **(Laura *et al.*, 2010).**

I-4-2-1- Anthocyanin

They are flavonoids due to the C6-C3-C6 carbon skeleton in their molecules, derivatives of the flavylium cation found in the oxo or carbonium forms, their huge diversity resulting from the many potential attachment sites for functional-methoxy and hydroxyl-groups in the cation ring (**Fig.06)**. In food, they are mainly found as anthocyanidin mono- di- and triglycosides **(Joshi et Goyal, 2011)**. Anthocyanins are responsible for colours ranging from pale pink to red to purple and deep blue (**Dahmoune *et al.*, 2013**).



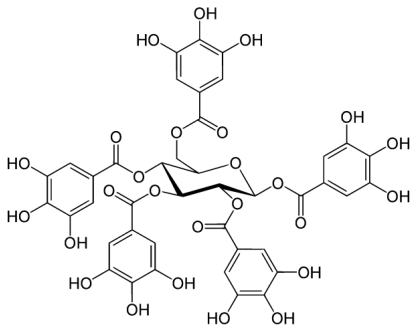
Anthocyanin	R3'	R5'	R3	R5
Pelargonidin 3-glucoside	H	H	Glc	OH
Pelargonidin 3,5-diglucoside	H	H	Glc	Glc
Cyanidin 3-glucoside	OH	H	Glc	OH
Cyanidin 3,5-diglucoside	OH	H	Glc	Glc
Delphinidin 3-glucoside	OH	OH	Glc	OH
Delphinidin 3,5-diglucoside	OH	OH	Glc	Glc

***Glc** : Glucose.*

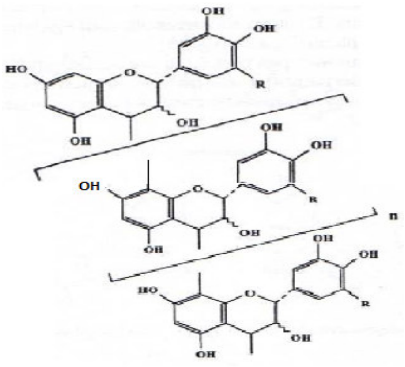
Figure 06: Chemical structures of the anthocyanins of pomegranate juice. (**Menna Parreno, 2013**).

I-4-3- Tannins

Tannins are complex phenolic compounds obtained from the condensation of simple phenols. They are divided into two groups: hydrolysable tannins (carbohydrate ester and phenolic acids) and condensed tannins (dimers, oligomers and/or polymers of flavannes-3-ols or flavannes -3, 4-diols) (**Fig.07)**.(**Macheix *et al.*, 2005 ; Makkar, 2003**).



a: Hydrolysable tannins.



b: condensed tannins.

Figure 07: Chemical structure of tannins (**Macheix *et al.*, 2005**).

- Abundant tannins in pomegranate are ellagitannins, these compounds are hydrolyzing tannins exhibiting high reactivity, in the center of their molecule containing a monosaccharide (most often glucose), whose hydroxyl groups are partly or completely esterified by gallic or ellagic acid radicals. **(Sepulveda *et al.*, 2011; Sibel et Jale, 2012)**. Contents of these compounds in pomegranate fruits include 1500 – 1900 mg/L punicalagin (cv.Wonderful), while ellagitannins with ellagic acid are found at 2020 – 2660 mg /L. and 5700 mg/L. **(Larrosa *et al.*, 2010).**

I-5- Biological activities

Pomegranate has been used for thousands of years to cure a wide range of diseases across different cultures and civilizations. It has great nutritional values and numerous health benefits. Pomegranates as a treatment for cancer, Osteoarthritis and other diseases **(Bhowmik *et al.*, 2013).** Phenolic acids, flavonoids, and tannins are present in different parts of pomegranate fruit and this may be one of the reasons why many of the studies demonstrated that combinations of pomegranate extracts from different parts of the fruit were more effective than a single extract **(Jain *et al.*, 2014).** The table below (**Table.02**) summarizes some of biological activities of pomegranate juice.

Table 02: Effect of pomegranate juice on biological targets involved in some diseases.

Treatment	Results	Reference
Cardiovascular diseases		
330 mL/day for healthy patients (n = 51), 4 weeks	Decrease in blood pressure	**(Lynn *et al*., 2012)**
240 mL/day, Methabolic syndrome, (n = 30) ,4 weeks	Improvement in endothelial function	**(Hashemi *et al*., 2010)**
240 mL/day, Coronary ischemia (n = 45), 4 weeks.	Decrease in myocardial ischemia.	**(Sumner *et al*., 2005)**
50 mL/day healthy patients (n = 13), 2 weeks	- Decrease in Aggregation and retention of LDL - Decrease in the activity of PON-1 (20%)	**(Aviram *et al*., 2000)**
Different form of cancer		
250 mL/day, Man (n = 46), 15 - 54 months	Increase in antioxidant and antiproliferative capacities of plasma (prostate)	**(Pantuck *et al*., 2006)**
20 % of PJ in water, Rats, 10 weeks	- Decrease in the impact of aberrant crypt - Increase in Hepatic GST activity (colon)	**(Boateng *et al*., 2007)**
Obesity and Diabetes		
120 mL/day of PJ, Obese (n = 10) 1 month.	Stability in body weight and fat	**(Gonzalez-Ortiz *et al*., 2011)**
50 mL/ day of PJ, Diabetic (n = 20), 3 months	- Serum lipid peroxides - Increase in Glutathion and decrease in Ox-LDL macrophagiques	**(Rosenblat *et al*., 2006)**
40 g/day of PJC, Diabetic (n = 22), 8 weeks	Decrease in TG, LDL-c, LDL-C/HDL-c, TC/HDL-C	**(Esmaillzadeh *et al*., 2004)**
Antibacterial activity		
3 concentration of methanolic PPE: 4 mg/ml, 8mg/ml and 12mg/ml	Antibacterial activity against *S. aureus* (7.5, 11.5, 12.5 mm)and *S.epidermidis* (11.5, 13.5, 13.5mm) respectively.	**(Abdollahzadeh *et al*., 2011)**

II- Anthocyanin degradation in pomegranate juice:

II-1- Factors affecting the stability of anthocyanins:

Anthocyanins are highly unstable and very susceptible to degradation. Their stability is affected by several factors such as pH, temperature, the chemical structure, light, oxygen, the presence of enzymes, flavonoids, proteins and metal ions (**Castaneda - Ovando *et al.*, 2009**). For that, intense research has been done on stabilisation of anthocyanins and elucidation of the high stability of the color. (**Troise et Fogliano, 2013**).

II-1-1- Temperature:

Studies on the effect of temperature on anthocyanin, have indicated that the stability is dependent on the structure of anthocyanin, with the sugar moiety playing a significant role (**Lee et Khng, 2002**). Monomer anthocyanins and the corresponding colorant intensity decreased with the time–temperature combination, whereas the polymer fraction (brown pigments) exhibited the reverse (**Francis, 1995**). Table 03 shows the effect of thermal treatment on the stability of some pomegranate juice components.

Table 03: Effect of thermal treatment on the stability of some pomegranate juice components.

Parameters	Concentration	Reference
Pasteurisation 65 °C / 30 s Pasteurisation 90 °C/5 s	3% loss in red color of pomegranate juice. 22% loss in red color of pomegranate juice	**(Vegara *et al.*, 2013)**
Pasteurisation 90 °C for 5 h Pasteurisation 60,70, 80 °C/5 h	- Loss ranging from 76% to 87% of the initial anthocyanins levels in the juices. - Loss of anthocyanins from 95 mg/L to 46.07, 44.24, 24.41 mg/L respectively.	**(Ulrike *et al.*, 2013)**
Pasteurisation at 70, 80, 90 °C/ 15 - 90 min	Loss in ascorbic acid from (0,173 - 0,138 mg/mL), (158 - 122 mg/mL), (0,116 - 0, 086 mg/mL) respectively.	**(Ranu et Uma, 2011)**
Pasteurisation at 65, 80, 95 °C for 30 s Pasteurisation 65, 80, 95 °C for 60 s	- Increase in total anthocyanins from 109.3 to 123.2, 172.5, and 182.9 mg/L respectively. - Increase in total anthocyanins from 109.3 to 117.2, 167.1, and 159.8 mg/L respectively.	**(Mena *et al.*, 2013)**

II-1-2- Interaction with Vitamin C

Ascorbic acid can have several roles in the stability of the anthocyanin color. (**Markakis, 1982; Talcott *et al.,* 2003**). The presence of this molecule has shown a negative impact on anthocyanin stability, leading to the mutual degradation of these compounds (**Rodriguez-Saona *et al.,* 1999; Garzon et Wrolstad, 2002**). The anthocyanins decomposition is accelerated by the presence of ascorbic acid which can generate hydrogen peroxide, generator damaging free radicals to anthocyanins which, by polymerization can give a brown resinous precipitate (**Rein, 2005**). Conversely, the stability of acylated anthocyanin is increased in the presence of ascorbic acid and in addition, they can be protected against enzymatic degradation (**Rein, 2005**). It is assumed that condensation of anthocyanins with flavonols prevents formation of complexes between anthocyanins and ascorbic acid (**Skrede et Wrolstad, 2002**), probably by competition with the anthocyanins in the preference for condensation reactions. (**Mercadante et Bobbio, 2008**). The deteriorating effect is most pronounced when both oxygen levels and ascorbic acid concentrations are high. The reactions are known to be also accelerated by copper ions (**Fig. 08**). (**Skrede et Wrolstad, 2002**).

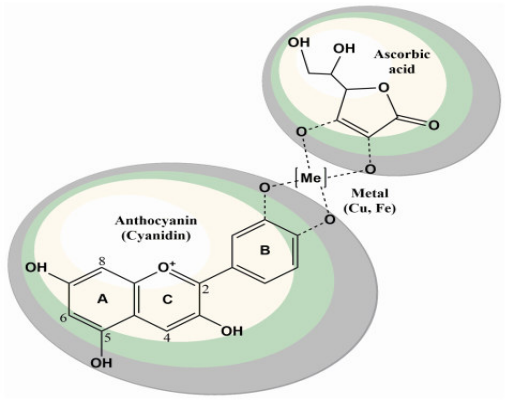


Figure 08: Suggested mechanism for the formation of the complex anthocyanin–metal–ascorbic acid (**Delgado-Vargas et Paredes-López, 2003**).

II-1-3- pH

Anthocyanins in aqueous medium behave for most of them as true pH indicators, at pH 1 and below, the anthocyanin pigment gives an intense red but becomes colourless or purple when the pH is increased to between 4 and 6. Meanwhile, the pigment turns a deep blue when the pH is between 7 and 8. Further increase in pH sees the anthocyanin pigment turning from blue to green and then to yellow. Such variation in colour has been attributed to structural transformation in response to changes in pH (**Clifford, 2000**). These color changes

are due to equilibrium reactions between different structural forms depending on the pH (**Fig. 09)**.

- At pH 1, the flavylium cation (red) is the predominant species and contributes to purple and red colors.
- At pH 2 – 4 blue quinoidal species are predominate .
- At pH values between 5 and 6, two colorless species is observed, a pseudo base carbinol and chalcone .
- At pH > 7, anthocyanins are degraded by their substituent groups (**Neill, 2002; Cavalcanti *et al.,* 2011**) .

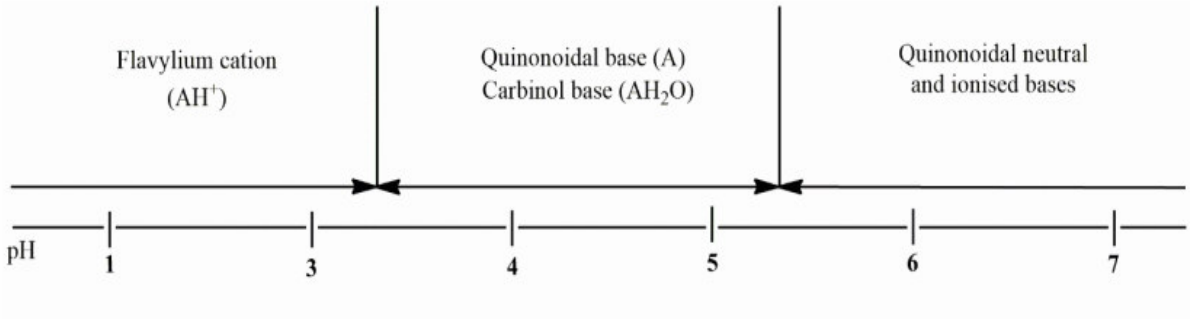


Figure 09: Chemical reactivity of the anthocyanins depending on the pH (**Clifford, 2000**).

II-1-4- Light

Light affects the anthocyanins in two different ways: it is essential for their biosynthesis, but also accelerates their degradation. Anthocyanins preserve their color better when kept in the dark; the difference was already seen after 24 hours when anthocyanins were stored in the light. The stability of anthocyanin in storage is affected by light and especially in the presence of sugar (**Cavalcanti *et al* ., 2011**)

II-1-5- Oxygen

Anthocyanin content decreased for all atmospheres tested; however, high oxygen (> 21 kPa) caused a higher decrement (**Gonzalez-Aguilar *et al.,* 2010**). Oxygen has a deleterious effect on anthocyanins, and it is known that anthocyanins stored under vacuum, nitrogen or argon atmosphere are more stable than anthocyanins exposed to molecular oxygen. The oxygen may degrade anthocyanins either directly or indirectly by oxidizing compounds, which in turn, may degrade the anthocyanins; this deteriorating effect is most pronounced when both oxygen levels and ascorbic acid concentrations are high (**Skrede et Wrolstad, 2002**).

II-1-6- Self-association

Stability of anthocyanins can be attained by self-association (**Fig. 10)**, that is, when two or more anthocyanin molecules are associated in relatively concentrated (> 1 mM) solutions (**Mercadante et Bobbio, 2008; Pina *et al.*, 2012**).

This effect was verified by increasing the concentration of the cyanidin 3,5-diglucoside solution from 10^{-4} M to 10^{-2} M with a consequent bathochromic shift in maximum wavelength absorption in the visible region (**Mercadante et Bobbio, 2008; Timberlake et Bridle, 1975**).

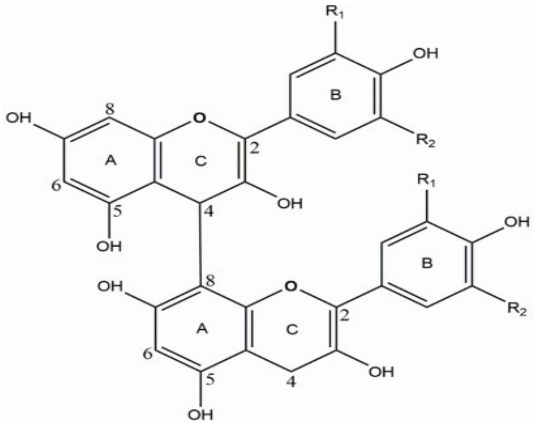


Figure 10: Self-association of anthocyanins at the C-4 position (**Giusti et Wallace, 2009**).

III- Yoghurt

III-1- Definition and classification

Yogurt is a product made from heat treated milk that may be homogenized prior to the addition of lactic acid bacteria cultures containing *Lactobacillus bulgaricus* and *Streptococcus thermophilus* (Code of Federal Regulations Section 131.203, 2011). Similarly, **Tamime (2002),** defined yoghurt as a product of the lactic fermentation of milk by addition of a starter culture, which results in a decrease of milk pH to less than or equal to 4.6. Industrially, yoghurts can be largely divided into two types. A set-style yoghurt is made in retail containers giving a continuous undisturbed gel structure in the final product, on the other hand, stirred yoghurt as a delicate protein gel structure that develops during fermentation (**Tamime et Robinson, 1999**).

- Yoghurts come in a variety of textures (e.g. liquid, set, and smooth), fat contents (e.g. luxury, low-liquid, virtually fat-free) and flavors (e.g. natural, fruit, cereal), can be consumed as a snack or part of meal, as a sweet or savory food, and are available all year round. This

versatility, together with their acceptance as a healthy and nutritious food, has led to their widespread popularity across all population subgroups (**Mckinley, 2005**).

III-2- Health Benefits

According to the available literature, it can be suggested that the health benefits associated with yoghurt consumption is well known for centuries. Yoghurt is considered as healthy food due to its high digestibility and bioavailability of nutrients and also can be recommended to the people with lactose intolerance, gastrointestinal disorders such as inflammatory, bowel disease, and aids in immune function and weight control.

- For instance, milk products including yoghurt is a rich source of calcium in bio-available form which is reported to provide 41% of the recommended daily requirement of Calcium for a 5-year old through a serving of 50 g of yoghurt. (**Lourens-Hattingh et Viljoen, 2001; Mckinley, 2005**). Yoghurt acts as a probiotic carrier food that is considered as an easy food to incorporate probiotics which results high probiotic viability. Bio-yoghurt is considered to be an ideal source for the delivery of viable probiotic strains*, Lactobacillus acidophilus* and *Bifidobacterium bifidum* which are the most common probiotics used in the dairy industry. However, in order to attain the probiotic effect, it is reported the need of consuming adequate amounts of viable probiotic cells regularly which is known as the therapeutic minimum. Therefore, the consumption should be more than 100 g of bio-yogurt containing more than 106 CFU/mL viable cells (**Mckinley, 2005**).

Material and methods

II- Materials and methods

II-1- Chemicals

All solvents and reagents used were of analytical grade. Sodium acetate ($CH_3CO_2{\cdot}3H_2O$), Potassium chloride (KCl), Sodium hydroxide (NaOH), Aluminium chloride hexahydrate ($AlCl_3{\cdot}6H2O$), were supplied from Biochem-chemopharma (Quebec, Canada). Folin–Ciocalteau's phenol reagent, 1,1-diphenyl-2-picryl-hydrazil (DPPH) were purchased from Sigma-Aldrich (Germany). Gallic acid and rutin were supplied from Biochem-chemopharma (UK), Ascorbic acid was provided from Sigma-Aldrich (USA). Hydrochloric acid fuming 37% GR (HCl), was purchased from Merck, Germany, Citric acid was supplied from Biochem-chemopharma (USA).

II-2- Plant material

Pomegranates (*Punica granatum L.*) were harvested in December 2015 at optimal maturity, which is defined by burgundy color of pomegranate. The harvest from Mitidja, Blida (Algeria), involved a random sampling from 10 trees. Before juice extraction, pomegranate fruits were washed with tap water and damaged fruits were discarded. Arils of fruits were hand-separated and pressed using a domestic juicer (Moulinex masterchef 370. France). The fresh juice was filtered through a sieve to remove pulp and seeds. The freshly pressed juice was packed in HDPE (High Density Polyethylene) plastic bottles of 330 cl capacity and frozen at -20 °C.

II-3- Thermal treatment

For the thermal processing, frozen juice samples were thawed to room temperature (T° = 23 ± 1 °C) (**Fig. 11**). After homogenisation, Ascorbic Acid Fortified Juice with 100 mg L^{-1} (AAFJ-100) and 200 mg L^{-1} (AAFJ-200) were prepared from the squeezed thawed juice, which represents the Non-Fortified Control Juice (NFCJ). The three types of juice were equally divided (8 mL) into sealed Pyrex tubes (100 mm × 12 mm). The juice samples were pasteurised at four different isothermal conditions (60, 70, 80 and 90 °C) for different holding times (**table 04**) by immersion of sealed Pyrex tubes in a thermostatic and stirred water-bath (Memmert type-ONE 7, Schutzart DIN EN 60529-IP 20. Germany) previously set at the desired temperature.

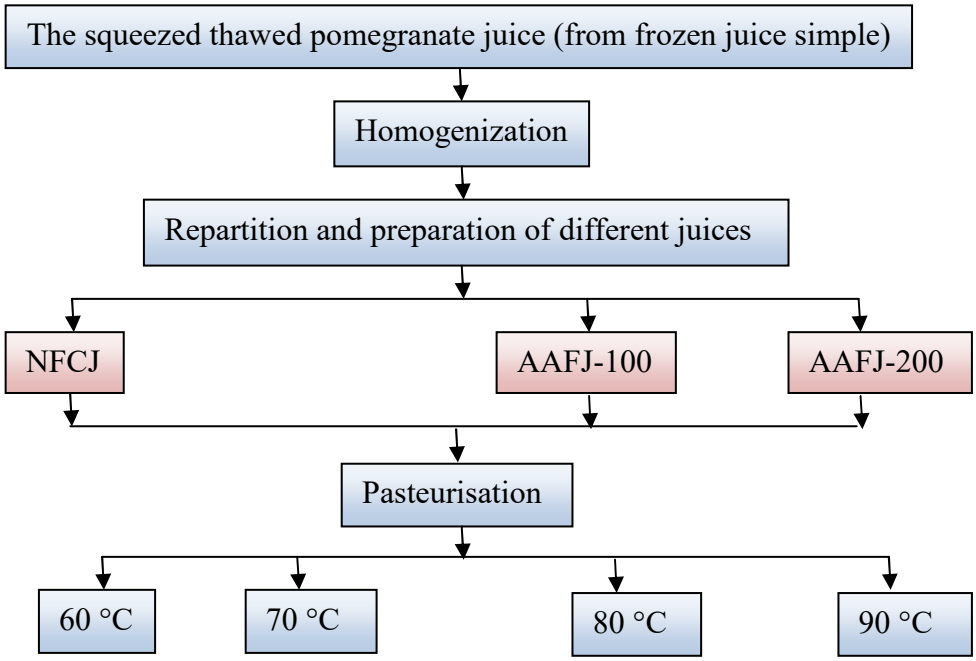


Figure 11: Experimental flowchart prior to thermal treatment studies.

Table 04: Different time and temperature profile of thermally pasteurized pomegranate juice.

Thermal treatment	
T (°C)	**t (min)**
60	0 15 60 120 180
70	0 90 150 180
80	0 10 20 30 60 180
90	0 5 15 30 60 180

- Juice temperature variations (ΔT (t), °C) of the cold spot (slowest heating point) within a sealed Pyrex tube (located at the middle of the thermostatic water-bath) were recorded every five seconds by a Type-K external thermocouple probe. The external thermocouple probe is placed at the geometrical centre (considered to be coldspot or slowest heating point) of one tube and connected to a probe of temperature to retrieve the time-temperature profiles (T (t)), also called heat penetration data. After each temperature-time processing, Pyrex tubes (three replicates at same time, randomly chosen) were immediately cooled down in ice water bath to minimize the thermal effect during cooling. The thermal processed juices stored in amber sealed vials of 20 mL capacity volume and kept frozen (at -20 °C) until analysed as described below.

II-4- Analytical methods

The samples (in triplicates) of the freshly pressed and/or thermal processed juice; at different isothermal conditions (60, 70, 80 and 90 °C) for different holding times; were analysed.

II-4-1- Determination of total dry matter

The total dry matter (TDM) determination of juice samples was carried out by the gravimetric method using the oven-drying method (**AOAC, 1998**). The juice samples were subjected to a drying temperature of T° = 103 ± 2 °C until constant weight and the results were expressed in percent dry basis (%, w/v) as following:

$$C(\%) = \frac{W_i - W_0}{W_i} \times 100$$

Where:

***M_C*:** Moisture content;
***W_i*:** the loss in weight (g) on drying;
***W_0*:** the initial weight of sample (g).

TDM = 100 – MC

II-4-2- Colour intensity

For the freshly pressed juice; a direct absorbance (A) measurement at 420, 520, and 620 nm was carried out using UV-visible spectrophotometer (UV-VIS Spectrophotometer UV-9200, Biothech Engineering Management CO.,Ltd. UK). The following

spectrophotometric attributes were calculated; juice colour intensity (JCI) as the sum of A420 nm, A520 nm and A620 nm; tint as the ratio of A420 nm to A520 nm; color proportion of blue (Bl %), yellow (Ye %) and red (Rd %) were calculated by dividing A420 nm, A520 nm and A620 nm, to JCI, respectively (**Glories, 1984; Kelebek *et al*., 2008**):

$$JCI = \sum \left(A_{420nm} + A_{520nm} + A_{620nm} \right)$$

$$\%\,\text{blue} = \left(\frac{A_{420nm}}{CI} \right) \times 100$$

$$\%\,\text{yellow} = \left(\frac{A_{520nm}}{CI} \right) \times 100$$

$$\%\,\text{red} = \left(\frac{A_{620nm}}{CI} \right) \times 100$$

$$Tint = \frac{A_{420nm}}{A_{520nm}}$$

II-4-3- Titratable acidity and pH

Total titratable acidity (TA) of juice samples was determined potentiometrically by titration with 0.1 N sodium hydroxide (NaOH) until pH endpoint of 8.2. Results are expressed as "g citric acid/ L juice" and calculated as follows (**AOAC, 1998**):

$$C_{\text{citric acid}} \times V_{\text{citric acid}} = C_{\text{NaOH}} \times V_{\text{NaOH}}$$

$$\mathbf{m_{citric\ acid}\ (g/100\ ml\ of\ juice)} = 0.064 \times V_{\text{NaOH}}$$

Where:

C: concentration;

V: the volume in ml.

m: the mass in grams;

- pH measurements (at T° = 20 ± 1 °C) were performed using a pre-calibrated microprocessor pH meter (HANNA pH-211 HI1332, Romania).

II-4-4- Total soluble solids measurement

Total soluble solids (TSS) content of juice samples was measured at T° = 20 ± 1 °C with an Abbe-type refractometer (AR 12, SCHMIDT+HAENSCH GmbH & Co., Berlin, Germany) and the results were expressed as °Brix.

II-4-5- Dissolved oxygen content

For the freshly pressed juice, the dissolved oxygen content was measured with an Oxi 730 oxygen electrode, equipped with Cell 325 probe and an Oxicall-SL air calibration beaker (WTW Wissensschaftlich Technishe, Weinheim, Germany).

II-4-6- Total phenolic content

The amount of total phenolic compounds (TPC) in the prepared juices (NFCJ, AAFJ-100, AAFJ-200) was determined using Folin-Ciocalteu method (**Georgé *et al.*, 2005**). This method is based on reducing, in the alkaline medium, phosphotungstic mixture $H_3PW_{12}O_{40}$ and $H_3PMo_{12}O_{40}$ of Folin reagent, by the oxidizable group of phenolic compounds, leading to the formation of reduction products of blue color (**Enneb *et al.*, 2015**). Thus, a 2.5 mL sample of water- diluted Folin-Ciolcateu reagent (1/10) was added to the juices. The mixture was incubated for 2 min at room temperature, and 2 mL of sodium carbonate (75 g/L) was added. The mixture was incubated for 30 min at room temperature. The specific absorbance at 750 nm was immediately measured, using UV-Visible light spectrophotometer (UV-VIS Spectrophotometer UV-9200, Biothech Engineering Management CO.,Ltd. UK). TPC concentration was calculated from a calibration curve, using gallic acid as a standard and the results were expressed as mg gallic acid equivalents in a liter of fruit juice (mg GAE/L of juice) (GAE/L J). All determinations were carried out in triplicate.

II-4-7- Total flavonoids content

The total flavonoids content (TFC) in the fresh pressed juice (NFCJ, AAFJ-100) was determined according to the mostly applied colorimetry method of **Bahorun *et al.* (1996)**, based on the formation of aluminium-flavonoid complexes (**Fig. 12**). The juices were diluted and 1.5 mL of 2% (w/v) aluminium chloride ($AlCl_3$) was added to 1.5 mL of diluted juices or rutin (positive control) and then mixed using vortex mixer (EV-102, tehtnica zelezniki, Germany) for approximately 10 s. The mixture was allowed to stand for 15 min. Absorbance of the mixture was determined at 430 nm versus the prepared blank using Uv-vis light spectrophotometer (UV-VIS Spectrophotometer UV-9200, Biothech Engineering

Management CO.,Ltd. UK). TFC was expressed as mg rutin equivalent per litre of juice (RE/ L J). Samples were measured in triplicate.

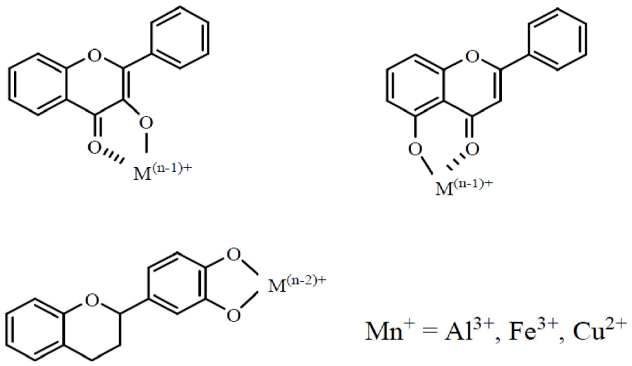


Figure 12: The chelation of metal ions by flavonoids (**Dangles, 2006**).

II-4-8- Radical-scavenging test

The radical-scavenging activity of NFCJ and AAFJ-100 was evaluated by the DPPH• assay. DPPH• (2,2-diphenyl-1-picrylhydrazyl) is a stable highly colored free radical (**Fig 13),** that can abstract labile hydrogen atoms from phenolic antioxidants (ArOH) with concomitant formation of a colorless hydrazine (DPPH-H). (**Malien-Aubert *et al.*, 2001**).

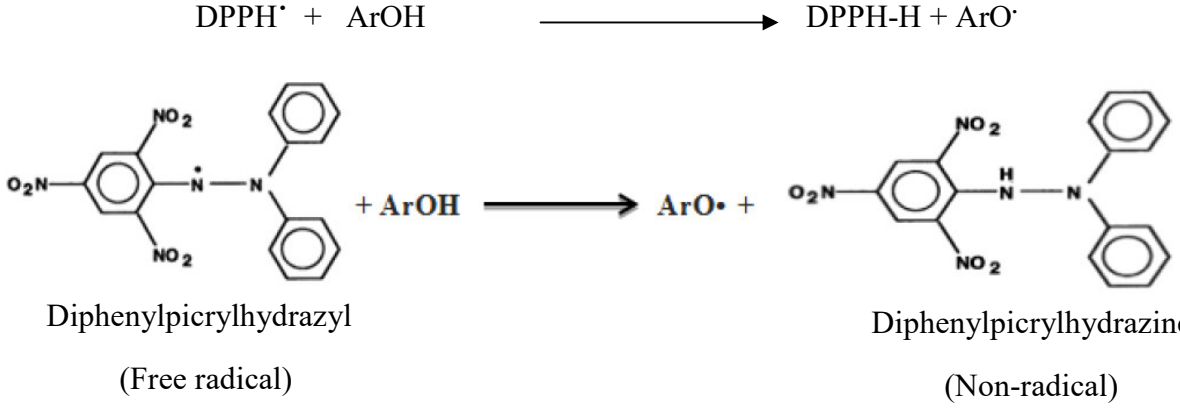


Figure 13: DPPH• Radical reduction (**Molyneux, 2004**).

- The free radical-scavenging activity (RSA) was measured following **Achat *et al.* (2012)** method. Two dilutions of the studied juices were prepared, one ml of each solution was added to 2 ml of DPPH• solution (2.10^{-4} mol/L in methanol) and the mixture was left in the dark at room temperature for 30 min. The absorbance was measured at 517 nm. The total

RSA of each juice was expressed as the percentage of DPPH˙ reduced and was calculated by the following equation:

$$RSA = \frac{(A_0 - A_1)}{A_0} \times 100$$

Where:

A_0: Absorbance of DPPH˙ solution without any antioxidant;

A: Absorbance of DPPH˙ solution after reaction with the juice.

All experiments were performed in triplicate.

II-4-9- Determination of total monomeric anthocyanin content

Total monomeric anthocyanins (ANC) content of juice samples was monitored by the pH differential method as outlined by **Lee *et al.* (2005).** Monomeric anthocyanin pigments reversibly change color with a change in pH; the colored oxonium form exists at pH 1.0, and the colorless hemiketal form predominates at pH 4.5. The difference in the absorbance of the pigments at 520 nm is proportional to the pigment concentration. (**Fig .14**) (**Lee *et al.*, 2005**).

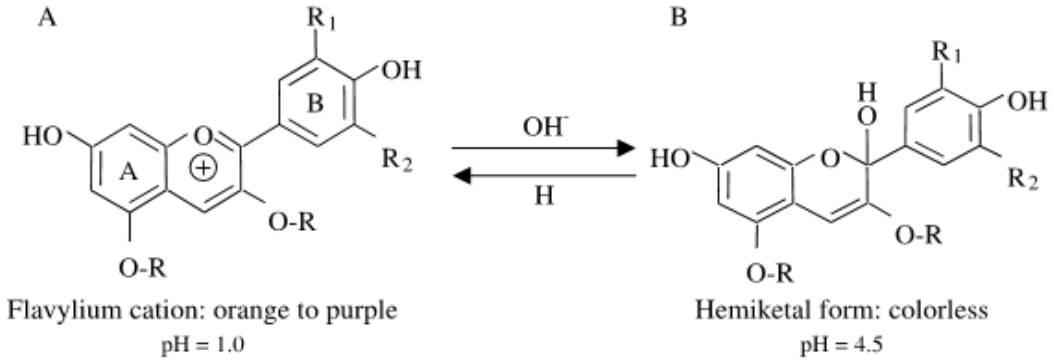


Figure 14: UV–Visible spectra of anthocyanins in pH 1.0 and 4.5 buffers, and the structures of the flavylium cation (A) and hemiketal forms (B). Glycosidic substituent. (**Lee *et al.*, 2005**).

- After dilution of the filtered juice samples with potassium chloride buffer (0.025 M, pH = 1.0) and sodium acetate buffer (0.040 M; pH = 4.5) and allowed to equilibrate for 20 to 50 min. The absorbance of equilibrated juice samples was measured versus a blank cell (filled with distilled water) for pH 1.0 and 4.5 at maximum absorbance wavelengths ($\lambda_{visile\ max}$ =520 nm) and at 700 nm to correct for haze. Measurements were performed in triplicates using UV-visible spectrophotometer (UV-VIS Spectrophotometer UV-9200, Biothech Engineering Management CO.,Ltd. UK). Results are expressed as cyanidin-3-glucoside basis. Degraded

anthocyanins in the polymeric form are resistant to color change regardless of pH and are not included in the measurements because they absorb at pH 4.5 as well as pH 1.0. Anthocyanin pigment concentration is calculated as follows:

$$\left[ANC\left(as\ mg\ \ Cyan\text{-}3\text{-}glucoide \right) \right] = \frac{A \times MW \times DF \times 10^3}{\varepsilon \times l}$$

Where: $A = \left(A_{520nm} - A_{700nm} \right)_{pH1.0} - \left(A_{520nm} - A_{700nm} \right)_{pH4.5}$;

MW (molecular weight) = 449.2 g/mol for cyanidin-3-glucoside (cyd-3-glu);

DF = dilution factor;

l = pathlength in cm = 1;

ε = molar extinction coefficient = 26 900 $L.mol^{-1}.cm^{-1}$, for cyd-3-glu;

10^3 = factor for conversion from g to mg.

II-5- Formulation of Flavoured yoghurt at laboratory scale

II-5-1- Manufacture of yoghurts

The preparation of yoghurt was made in the laboratory 3BS (University of Bejaia) respecting the diagram for making standard yoghurt with addition of pomegranate juice. The adapted recipe is the one determined within the work which is included in our research laboratory project. Thus, four steamed yoghurts were manufactured; cow milk was homogenized and heated to 95 °C for 5 min then cooled to 40 °C. After then, traditional starter culture was added and the mixture was incubated until the gel structure was formed. The gel was stored at refrigerator (6 ± 2°C), in this case a standard yoghurt was obtained. The same experiment was done with the other yoghurts except that non fortified pomegranate juice and fortified pomegranate juice with food additive E 300 (ascorbic acid at 100 ppm) and commercial pomegranate juices were added.

Table 05: Recipe of standard yoghurt and yoghurt flavored with the pomegranate juices.

Recipe	Milk (L)	Sugar (g)	Pomegranate juice (ml)	Lactic Ferment (%)
standard yoghurt	1	80 - 100	0	0.02
yoghurt with non fortified pomegranate juice	1	80 - 100	---	0.02
yoghurt with fortified pomegranate juice	1	80 - 100	---	0.02
yoghurt with commercial pomegranate juice	1	80 - 100	---	0.02

II-5-2- Physico-chemical properties of yoghurt

Physico-chemical properties of the manufactured yoghurts (standard yoghurt, yoghurt with non fortified pomegranate juice, yoghurt with pasteurized pomegranate juice at 60°C/15 min and yoghurt with commercial pomegranate juice) were determined namely, pH, dornic acidity, viscosity, the dry extract and fat contents (**Table 06**). These tests were carried out at the laboratory of the dairy industry "DANONE DJURDJURA".

Table 06: Physico-chemical properties of yoghurts

Measure	Method
pH	The pH value of yoghurt was measured at fixed temperature (9.5 - 10.5 °C) with a calibrated pH electrode (HANNA HI 2210).
Viscosity (g)	Apparent viscosity of yoghurt was expressed using a viscometer "TAXT EXPRESS" during 45 s.
Dornic acidity (°D)	10 g of sample (adjusted with distilled water up to 60 g), was put in acidometer apparatus then the result was directly displayed.
Brix degree	The soluble solids content of the filtered yoghurt (whey) was assessed by the refractometer, where sugar content value was given.
Total dry extract (%) Protein content (%) Fat contents (%)	50 g of yoghurt was placed in "Food scan" apparatus which give the values of total dry extract, protein and fat contents.

II-5-3- Microbiological analysis

Microbiological quality of prepared yoghurt was evaluated by enumerating total viable organisms. The organisms enumerated include total flora, yeast, moulds, total coliforms and specific bacteria of yoghurt **(Table 07).**

Table 07. Microbiological analysis of manufactured yoghourts.

Micro-organisms	Selective mediums	Incubation temperature	Incubation time	Method
Total Coliforms	VRBL	30 °C	24 h	3 g of the Yoghurt samples was spread plated in triplicates into prepared and dried petri-plates of suitable media for the enumeration of different organisms.
Total Flora	PCA	30 °C	72 h	
Yeasts, moulds	YGC	25 °C	5 days	
Streptococcus thermophilus	M17	37 °C	48 h	
Lactobacillus bulgaricus	MRS	37 °C	72 h	

VRBL: Violet Red Bile Agar

YGC: Yeast extract glucose chloramphenicol agar

M17: M17 agar

MRS: Rogoza and Sharpe agar

.

II-5-4- Antioxidant acitivity and ANC content of yoghurts

The anthocyanin content in yoghurts was determined using the pH differential methods (Section II-4-9). The Radical scavenging capacity was also measured in prepared yogurts by the $DPPH^{\bullet}$ assay (Section II-4-8). Ethanolic solution containing citric acid 1g L^{-1} was used as extraction solvent.

II-5-5- Sensory analysis

Evaluation of sensory properties of yoghurts (standard yoghurt, yoghurt with non fortified pomegranate juice and fortified pomegranate juice with food additive E 300 (ascorbic acid at 100 ppm) and commercial pomegranate juice) was studied. The panel was constituted by ten trained panellists from the staff members of the Life and Nature Science (University of Bejaia). Panelists evaluated the color, taste, texture, flavor and odor of each sample, using a numerical scale 1 - 5 (1 = not acceptable, 5 = extremely good).

Statistical analysis

All experiments were conducted in triplicate and results are expressed as mean ± standard deviation (SD). The analysis of variance (*ANOVA*) was performed using XLSTAT Release 10 (Addinsoft, Paris, France). Tukey's multiple range test (HSD) was used to compare means of the determined parameters. Evaluations were based on the $p < 0.05$ significance level.

Results and discussion

II-1- Physico-chemical characterization of fresh and fortified pomegranate juice

The initial physicochemical properties of the untreated pomegranate juice (PJ); Non-Fortified Control Juice (NFCJ) and Ascorbic Acid Fortified Juice with 100 mg/L (AAFJ-100) are summarized in Table 08.

Table 08: Main characteristics of pomegranate juices (PJ); Non-Fortified Control Juice (NFCJ) and Ascorbic Acid Fortified Juice with 100 mg /L (AAFJ-100).

Analysis	NFCJ	AAFJ-100
Juice yield (%)	66.76 ± 0.00^{a}	66.76 ± 0.00^{a}
pH	4.27 ± 0.00^{a}	4.25 ± 0.00^{b}
Total soluble solids (°Brix)	16.95 ± 0.07^{a}	16.2 ± 0.00^{b}
Titratable acidity * (g/100 g of juice)	0.17 ± 0.00^{b}	0.20 ± 0.01^{a}
Dissolved oxygen (mg/L)	$3.02 \pm 0{,}05^{a}$	$2.01 \pm 0{,}03^{b}$
Total dry matter (%)	15.41 ± 0.10^{a}	14.49 ± 0.05^{b}
Colour intensity	1.99 ± 0.02^{a}	2.02 ± 0.01^{a}
Tint	1.29 ± 0.01^{a}	1.29 ± 0.04^{a}
% yellow	32.56 ± 0.24^{a}	32.89 ± 0.73^{a}
% red	24.85 ± 0.27^{a}	24.13 ± 0.77^{a}
% blue	42.14 ± 0.16^{a}	42.38 ± 0.39^{a}
Anthocyanin ** (mg /L)	66.29 ± 1.17^{a}	68.45 ± 3.75^{a}
Radical scavenging activity (%)	59.03 ± 1.75^{b}	67.14 ± 0.54^{a}
Total phenolic content (mg/ L)***	5666.66 ± 222.61^{a}	5527.77 ± 78.57^{a}
Total flavonoids (mg/L)****	187.88 ± 2.52^{a}	191.19 ± 9.51^{a}

Values are the means of three determinations ± standard deviation Values with different letters (a-b) were significantly different (Tukey, $p < 0.05$) for the two types of juice.
** As citric acid equivalent*
*** As cyanidin-3-glucoside equivalent.*
**** As gallic acid equivalent.*
***** As rutin equivalent*

The results showed that ascorbic acid fortification of the pomegranate juice (PJ) has a significant difference ($p < 0.05$) in the physicochemical parameters in AAFJ-100 as compared to NFCJ. However, no observed significant difference for tint, anthocyanins, total phenolic content and total flavonoid content in both juices (**Table 08**).

The juice yield was about 66.76% which agrees with the results (29.73 - 64.42%) reported by **Hmid *et al.* (2013). Tehranifar *et al.* (2010)**, showed a less amount of juice content about 26.95 to 46.55%. These cultivars can be interesting for juice production industries.

The pH values obtained in the current study are greater than those reported by **Tehranifar *et al.* (2010)** on pomegranate cultivars grown in Iran (3.16 - 4.09). Statistical analysis revealed a significant difference ($p < 0.05$) between NFCJ and AAFJ-100 for pH and titratable acidity; this is likely due to the acidity of the ascorbic acid fortification.

The total soluble solids content was 16.95 °Brix for the NFCJ and 16.2 °Brix for AAFJ-100; these results were in agreement with those (10 - 19 °Brix) obtained by **Poyrazoglu *et al.* (2002)**; **Fadavi *et al.* (2005)**. The juice dry matter are close to that obtained by **Al-maiman et Ahmed, 2002** (13.73 - 16.35%). There is a significant difference ($p < 0.05$) between the NFCJ and AAFJ-100 in the total soluble solids content and total dry matter; this may be due to the non controlling of the pulp content in the pomegranate juice.

The results of anthocyanins (ANC) content were lower than the ones obtained for eight pomegranate cultivars widely grown in Turkey (**Çam *et al.*, 2009**), with anthocyanin values between 81 and 369 mg cyanidin-3-glucoside equivalent/L of juice (**Çam *et al.*, 2009**). But they were close to the range of results obtained by **Hmid *et al.*, 2013** (56.58 - 188.7 mg cyanidin-3-glucoside equivalent/L of juice) for eighteen pomegranates (*Punica granatum* L.) cultivars grown in Morocco.

Ozkan *et al.*, 2009, reported that the total monomeric ANC content of PJ obtained from nine registered cultivars ranged from 46 to 450 mg cyanidin-3-glucoside equivalent/L of juice , this result shows that total anthocyanins content of pomegranates strongly depends on the cultivars. The pomegranate juice fortified with ascorbic acid (100 mg/L juice) showed no significant difference in anthocyanin content in comparison with fresh juice; this shows that ascorbic acid has no effect on the concentration of anthocyanins.

The studied pomegranate juices showed a lower antioxidant activity percentage comparing with the results reported by **Cam *et al.*, 2009** (73 - 91.8%), but higher than the values reported by **Tehranifar *et al.* (2010)** on twenty pomegranate juices extracted from arils in Iran (15.59 - 40.72%). In another study, the antioxidant activity of Ganesh pomegranate cultivar of juice was reported as 69% **(Kulkarni et Aradhya, 2005**). There is a significant difference ($p < 0.05$) between the NFCJ and AAFJ-100 in antioxidant activity; this is due the additional antioxidant effect of ascorbic acid fortification.

The total phenolics content (TPC) of the studied pomegranate juice are higher than those found by **Gil *et al.* (2000)** of pomegranate juice from fresh arils produced from Wonderful cultivar

harvested in California as 2117 ± 95 mg gallic acid equivalent/L of juice and less than TPC obtained from eight pomegranate arils widely grown in Turkey (2083- 3436 mg GAE/L J) **(Çam *et al.*, 2009)**. **Hmid *et al.*, 2013** found an amount which ranges from 1385 to 9476 mg GAE/L J of local cultivars and foreign cultivars (1284 - 8295 mg GAE/L J of juice).

The TPC from PJ were higher than those of the other juices such as turnip juice (772 mg GAE/L J of juice , red grape juices (1728 mg GAE/L of juice and red wine (1869 mg GAE/L of juice) **(Gatti *et al.*, 2011).**

According to the results, total flavonoids content found in pomegranate juice of both samples are higher than those found by **Hmid et al. (2013)** which ranges from 14.446 - 56.989 mg rutin equivalent/L of juice).

The variation in the data comparison in the present work may be due to other factors such as the different pomegranate cultivars, the growing, climatic, varietal conditions, and sample extraction method used in the experiments.

II-2- Changes in physicochemical parameters during pasteurisation on the fresh and fortified pomegranate juices

II-2-1-Thermal treatment

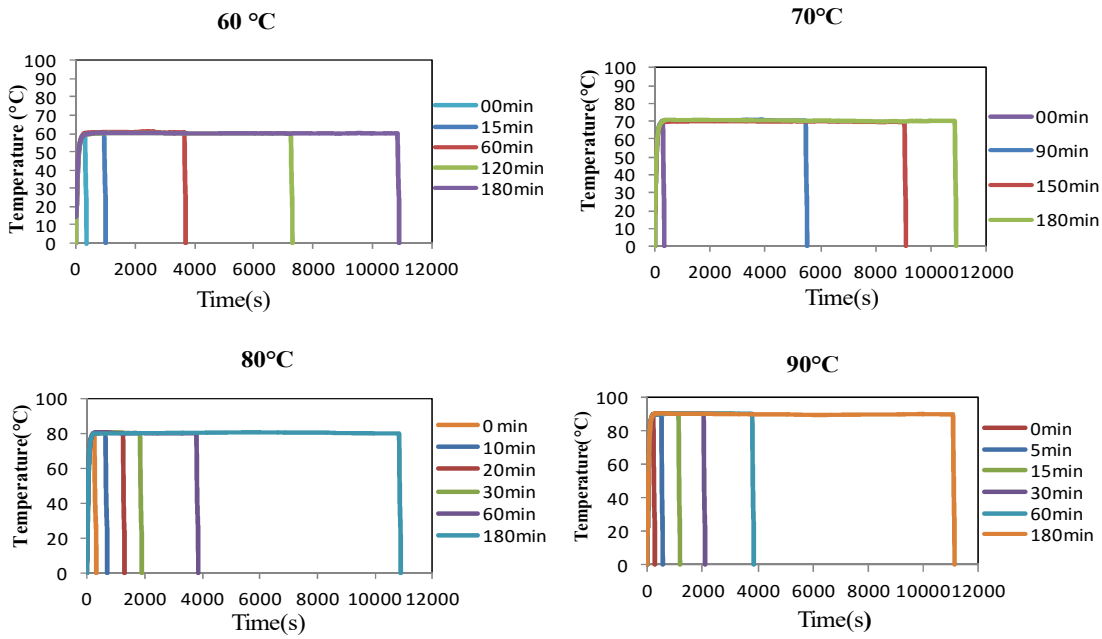


Figure 15: Time-temperature profiles, T (t), for the fortified and non-fortified pomegranate juice samples pasteurised at 04 isothermal temperatures (60, 70, 80 and 90 °C) in Pyrex sealed tubes.

Figure 15 shows the thermal history that explains the three typical stages of heat treatments. The recorded three stages thermal history included: (*i*) the heating up or coming-up stage, during which the retort reaches processing temperature; (*ii*) maintaining or set-up stage, during which the retort was held at the same processing-temperature level for a designated time and (*iii*) cooling dawn stage, during which the retort temperature was lowered to 25 °C.

The time for the juice to reach the temperature set up was below 4 min, and the cooling time was about 1 min. Then, the heat transfer time might be insignificant and treatment could be considered isothermal. The results of thermal history for fresh and fortified juices were identical, that's why the temperature is changing in the same way in both juices.

II-3- Changes in physicochemical parameters during pasteurisation

The effects of isothermal processing (pasteurisation) at different time and temperature (60 - 90 °C) profile on change of the different physicochemical properties from Non-Fortified Control Juice (NFCJ) and Ascorbic Acid Fortified Juice with 100 mg/L (AAFJ-100) are shown in (**fig 16 - 19**).

II-3-1-pH and titratable acidity change in fresh and fortified pomegranate juices

Fig.16 and **Fig.17** depict changes in pH from Non-Fortified Control Juice (NFCJ) and Ascorbic Acid Fortified Juice with 100 mg/L (AAFJ-100) during thermal treatment (pasteurisation) at 60 °C, 70 °C, 80 °C and 90 °C.

Organic acids play an important role in juices because of their influence on the organoleptic properties (flavour, colour, and aroma) as well as the stability and microbiological control of the products (**Mato *et al.,* 2005**). Citric acid is the predominant organic acid found in large quantity of pomegranate juice, followed by malic acid and very low amount of tartaric acid, as reported in other studies (**Gil *et al.,* 2000; Gundogdu et Yilmaz, 2012**).

According to the results, it is verified that the pH and titratable acidity (TA) presented a significant difference in time during thermal treatment, there was a decrease in some cases (90 °C/60 min) and an increase in others (80 °C/30 min) for the pH and (70 °C/90min), (60 °C/120 min) for the acidity, these data are in accordance with those reported by **Chen *et al.* (2013)**; **Fernandes *et al.* (2011)** for the TA but not the pH in some passion fruit juices .

This difference in the pH is probably due to a small variation in determination assays like the absolute homogeneity which cannot be achieved or may be the risk of evaporation caused by the opening pyrex tube containing the external thermocouple probe.

The decrease in TA could be attributed to the variation of pH and a chain degradation reaction of alpha hydroxy acid during the storage **(Chen *et al.*, 2013).** However, **Dahdouh (2015)** found that there is no definite relationship identified between pH and total titratable acidity.

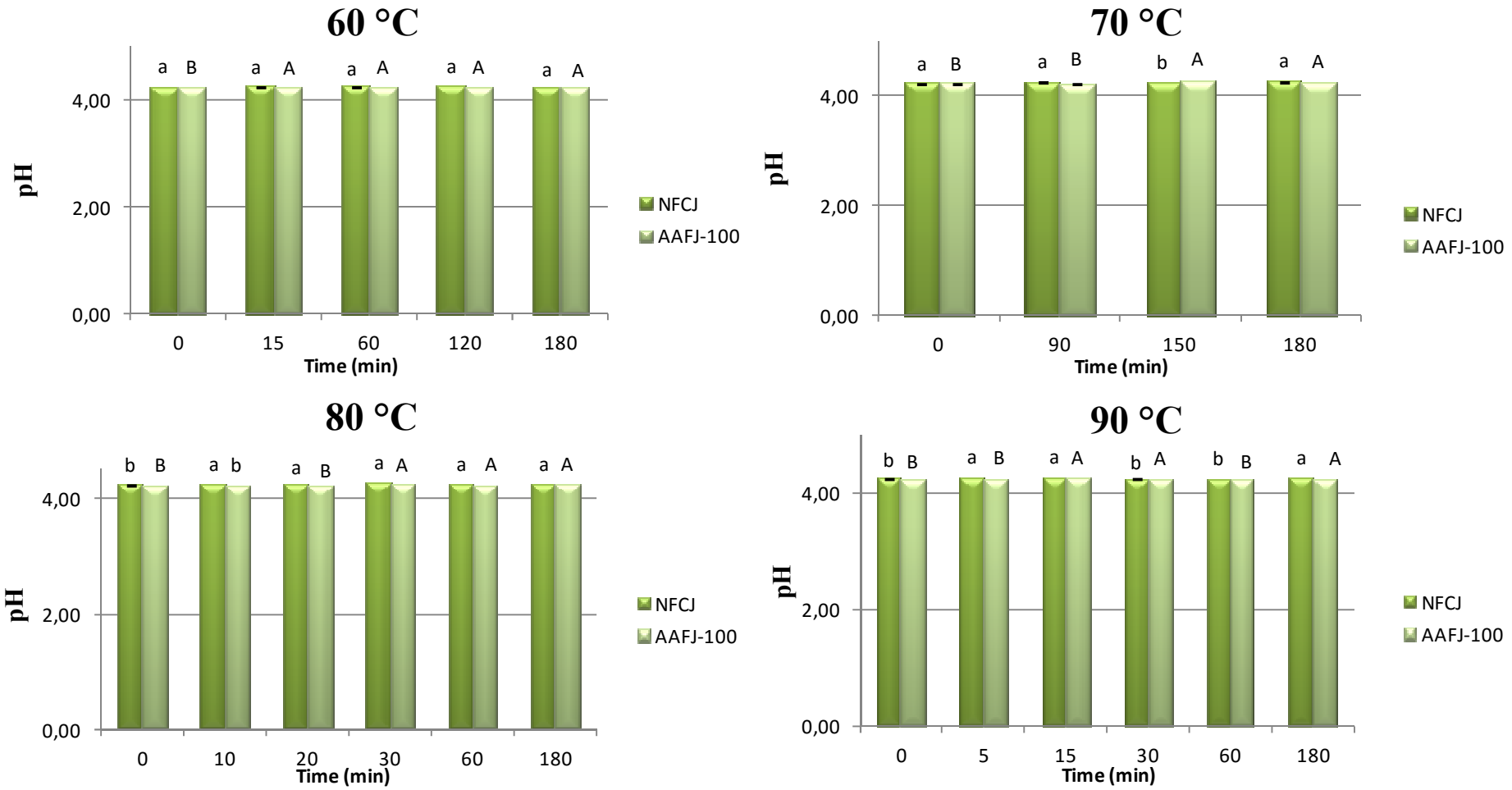


Figure 16: pH changes from Non-Fortified Control Juice (NFCJ) and Ascorbic Acid Fortified Juice with 100 mg/ L (AAFJ-100) during isothermal treatment at 60 °C, 70 °C, 80 °C and 90 °C.

Values are the means of three determinations ± standard deviation;
Values with different letters (a-b) were significantly different (Tukey, $p < 0.05$) for the two types of juice;
The results are ranked in decreasing order: a > b.

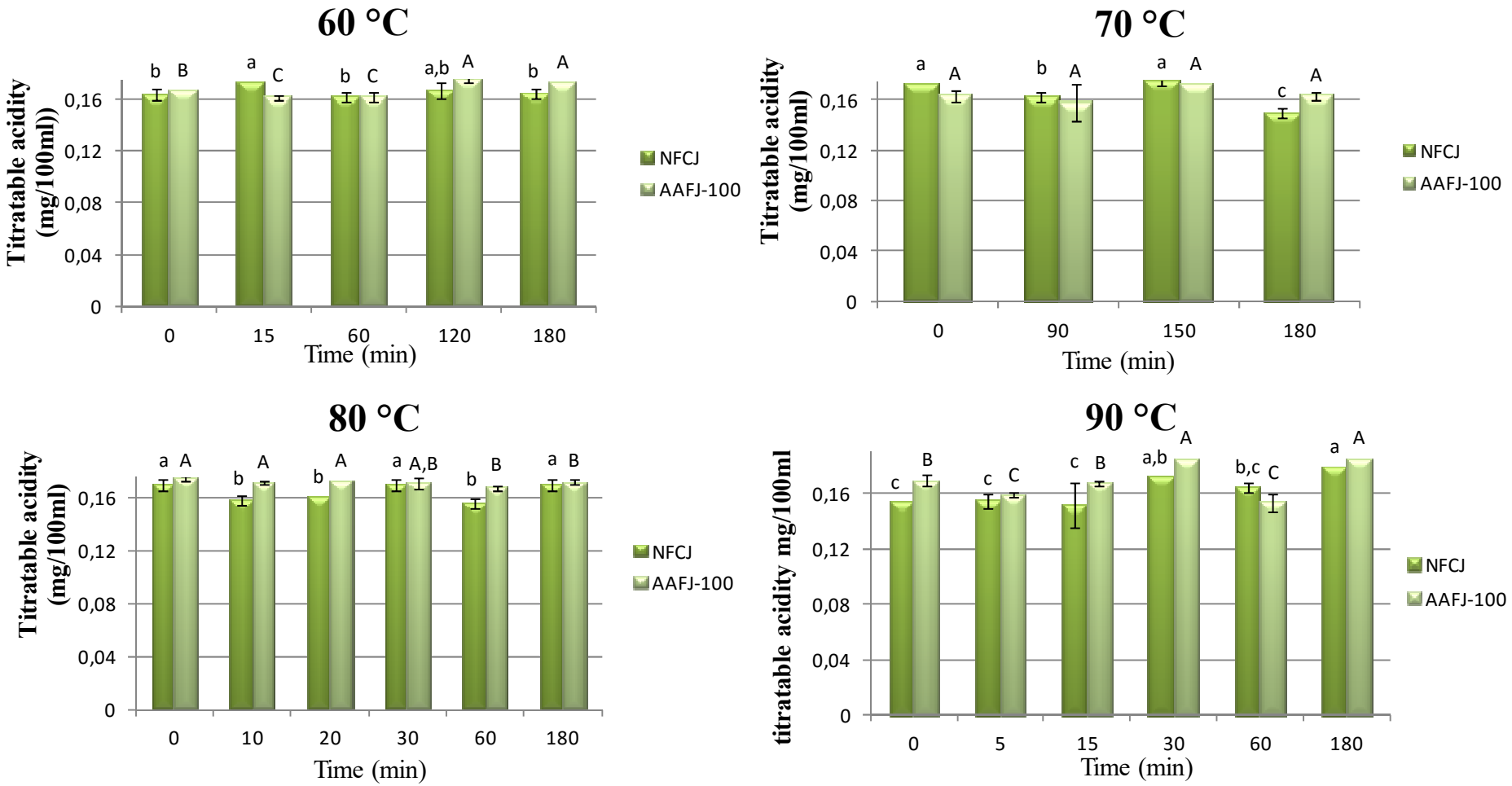


Figure 17: Changes in total titratable acidity from Non-Fortified Control Juice (NFCJ) and Ascorbic Acid Fortified Juice with 100 mg/L (AAFJ-100) during isothermal treatment at 60 °C, 70 °C, 80 °C and 90 °C

Values are the means of three determinations ± standard deviation;
Values with different letters (a-b-c) were significantly different (Tukey, $p < 0.05$) for the two types of juice;
The results are ranked in decreasing order: a > b > c.

II-3-2- Changes in total soluble solids (°Brix) and total dry matter from fresh and fortified juices

Changes in total soluble solids (TSS) and total dry mater (TDM) from Non Fortified Control Juice (NFCJ) and Ascorbic Acid Fortified Juice with 100 mg/L (AAFJ-100) during isothermal treatment (pasteurisation) at 60 °C , 70 °C , 80 °C and 90 °C are illustrated in **Fig 18-19**.

One of the basic criteria used for the definition of fruit juices is certainly Brix degree. As it is well known, Brix degree indicates the percentage of water-soluble solids in fruit juice and can be affected by many factors including variety, growth region, growth year and maturity level of the fruit. According to the European fruit juice association (AIJN proposal), the minimum Brix degree of pomegranate juice should be 14.0 (**Türkmen et Eksi, 2011**). Generally, the total soluble solids in the two pomegranate juice upon the application of the different time and temperature of thermal process were significantly affected.

For the total dry matter, there was a significant difference in time during thermal treatment. However, a decrease in dry matter was noticed (60 °C/15 min, 90 °C/ 15 min), which can be explained by the evaporation of some compounds in the drying (103 °C). An increase of the total dry matter was already observed in some cases (70 °C/90 min, 90 °C/15 min). This is probably due to the amount of collected pulp. According to **Tandon *et al*. (2003),** the higher soluble solids of pasteurised juice are due to water evaporation during thermal pasteurising in the steam kettle. **Rivas *et al.* (2006)** stated that the change in total soluble solids is due to the presence of the microorganisms that cause the fruit juice deterioration as a result of sugar fermentation.

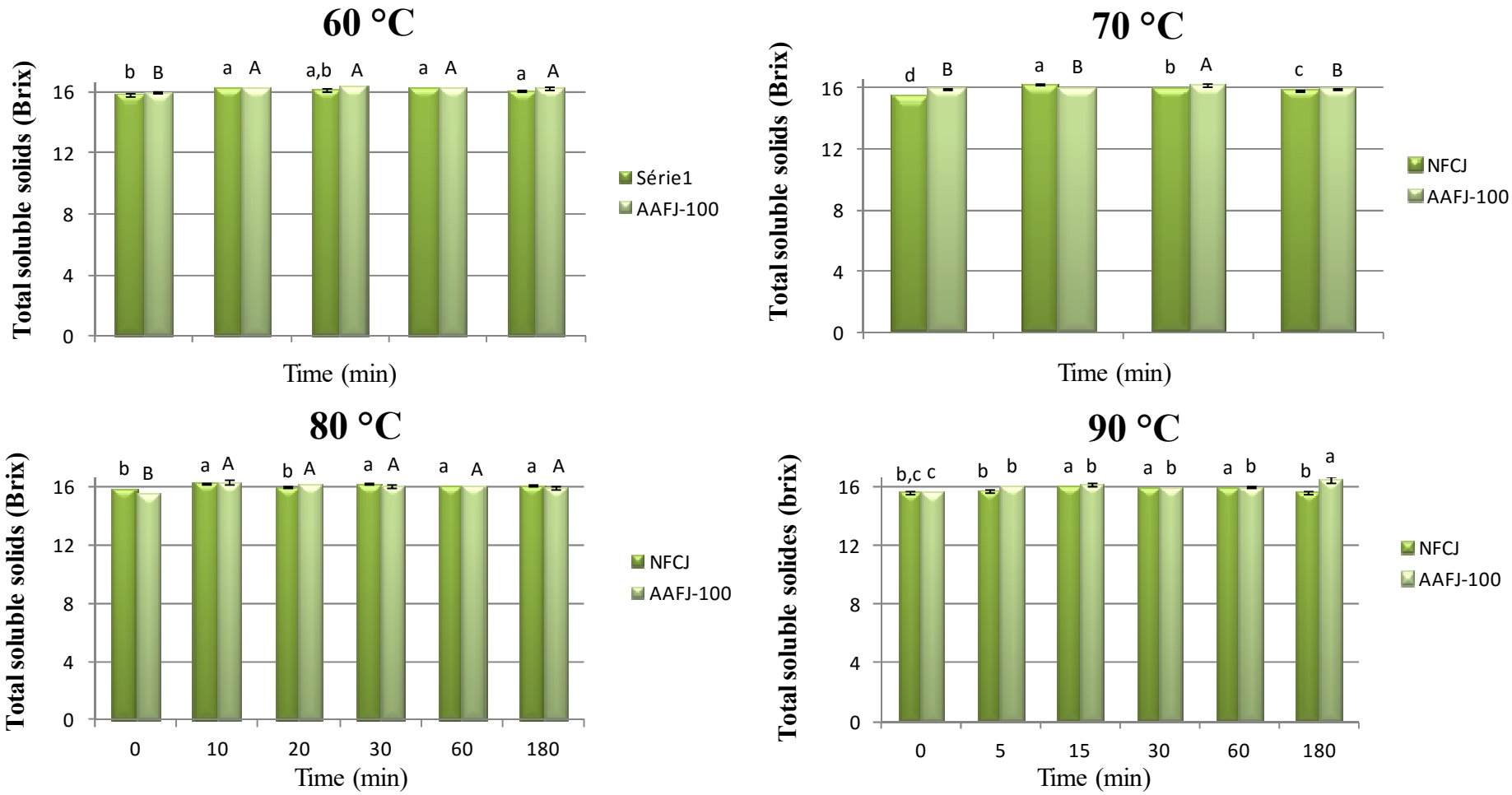


Figure 18: Changes in total soluble solids from Non-Fortified Control Juice (NFCJ) and Ascorbic Acid Fortified Juice with 100 mg L−1 (AAFJ-100) during isothermal treatment at 60 °C, 70 °C, 80 °C and 90 °C.

Values are the means of three determinations ± standard deviation;
Values with different letters (*a-b-c-d*) were significantly different (Tukey, $p < 0.05$) for the two types of juice;
The results are ranked in decreasing order: $a > b > c > d$.

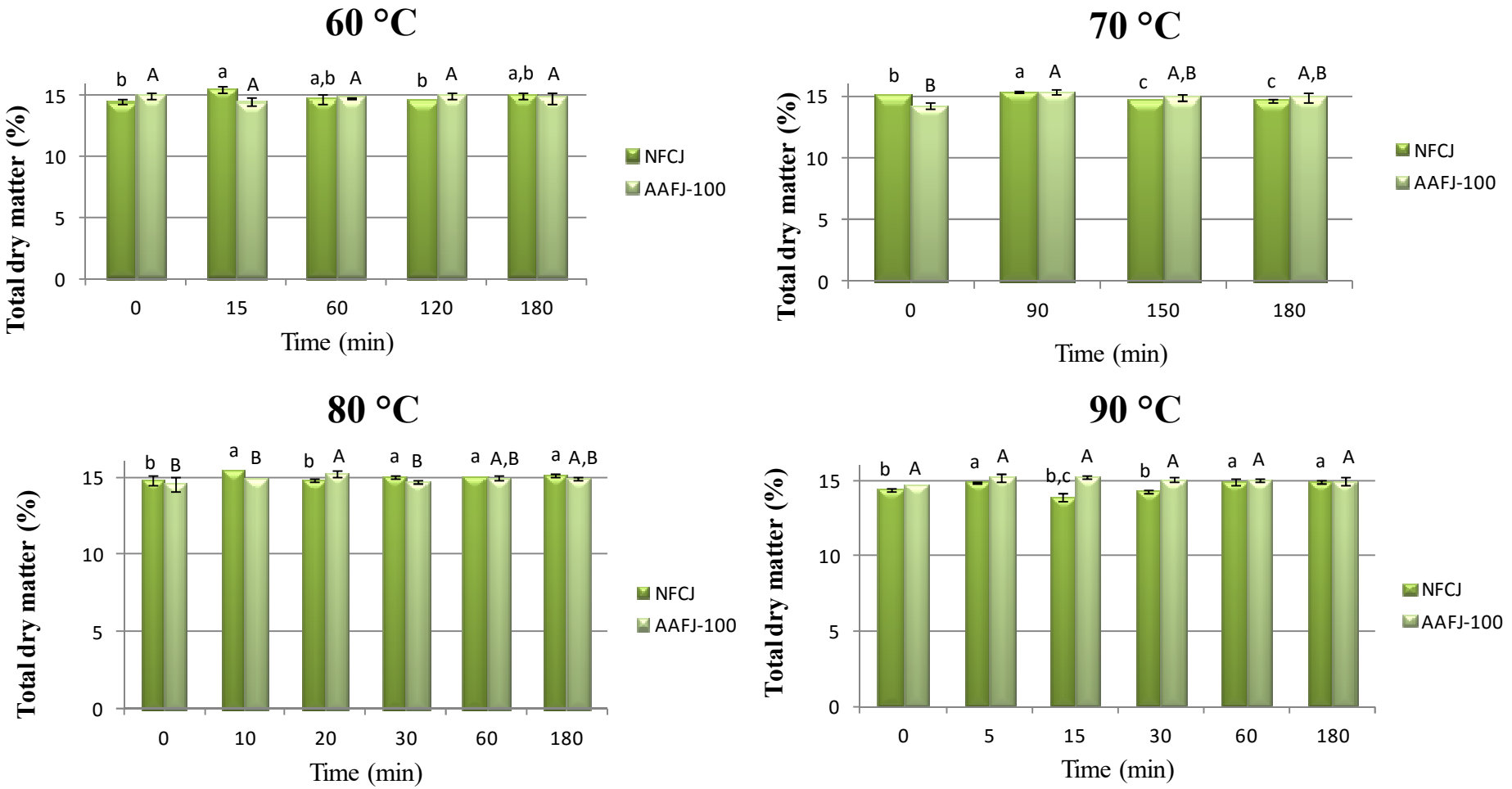


Figure 19: Changes in total dry matter from Non-Fortified Control Juice (NFCJ) and Ascorbic Acid Fortified Juice with 100 mg L−1 (AAFJ-100) during isothermal treatment at 60 °C, 70 °C, 80 °C and 90 °C.

Values are the means of three determinations ± standard deviation;
Values with different letters (*a*-*b*-*c*) were significantly different (Tukey, $p < 0.05$) for the two types of juice;
The results are ranked in decreasing order: $a > b > c$.

III- Changes in total monomeric anthocyanins during pasteurisation

The anthocyanin contents of all pomegranate juices (NFCJ, AAFJ-100 and AAFJ-200) during thermal treatment at different temperatures and time are shown in **fig.20** and **fig.21.** The results are represented as C/C_0 versus time, were C, is the ANC concentration at specified temperature and time, and C_0, is ANC concentration at t = 0 min for each temperature.

The anthocyanin retention is not affected by ascorbic acid fortification since no significant difference is noticed between NFCJ and AAFJ-100 but slightly affected by the increase in the concentration (AAFJ-200) of juices as illustrated in **fig.20.** These results highlight the destructive effect of ascorbic acid on the stability of anthocyanins as reported by **Nikkhah *et al.* (2010)**. Moreover **(Marti *et al.*, 2002),** reported that decomposition of anthocyanin is accelerated in the presence of ascorbic acid.

The presence of ascorbic acid (AA) has shown a negative impact on anthocyanin stability, leading to the mutual degradation of these compounds **(Rodriguez-Saona *et al.*, 1999; Garzo´n et Wrolstad, 2002; Brenes *et al.*, 2005).** However, **Veridiana *et al.* (2007)** reported that the anthocyanin degradation was not dose-dependent on the AA concentration**.** As it can be seen in **fig.21**, the ANC content decreased with time in all temperatures for all juices, we can also notice that there is no significant difference between 60 °C, 70 °C and between 80 °C, 90 °C for each pomegranate juice and the anthocyanins degradation is more pronounced with the severity of the temperature, this means that the ANC of pomegranate juice are sensible to the thermal treatment , the evaluation of these data demonstrated that the longer is the time, the higher is the temperature and the more important is anthocyanins degradation. **Chen *et al.*, (2013),** reported that anthocyanins were unstable at high temperatures.

Besides the ANC degradation to colourless forms, additional polymerization of ANC with themselves or with non-anthocyanin phenolics can occur, forming red to brown products at moderate temperatures **(Wrolstad *et al.*, 1990).**

The relative ANC retention for two types of pomegranate juice decreased significantly with increasing heating in a temperature interval from 60 to 90 °C, heating at the high-temperature long time resulted in total ANC losses ranging from 76% to 87% of the initial ANC levels in pomegranate juice **(Ulrike *et al.*, 2013)**.

On the other hand, it was reported that during short-term heating (for several minutes), even at 100 °C no considerable changes are observed in their contents or color of solutions **(Oszmian´ski, 2002; Pojer *et al*., 2013).**

Rommel *et al.* (1992) found that high-temperature, short-time (HTST) heat-treated blackberry juices retained most ANC. Similarly, **Mikkelsen et Poll (2002)** reported that much lower ANC losses occurred after HTST treatment of blackcurrant juice compared with low-temperature, long-time (LTLT) treatment. A plausible explanation for this is that during the LTLT treatment, enzymes that degrade ANC, including polyphenol oxidase, may be activated.

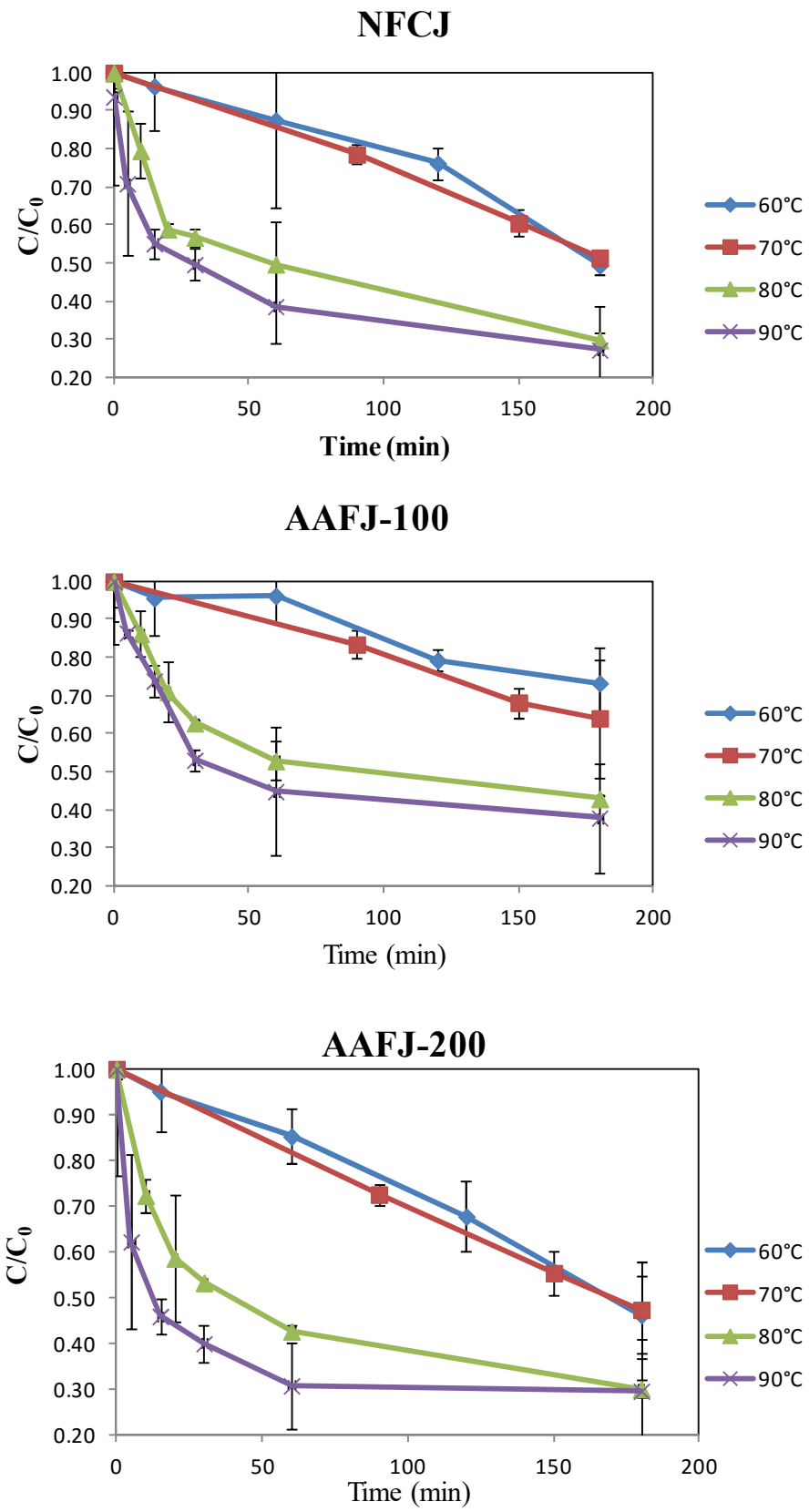


Figure 20: Anthocyanins kinetics degradation of Non-fortified control juice (NFCJ), Ascorbic acid fortified juice (AAFJ-100), and (AAFJ-200), during thermal treatment at temperature 60, 70, 80 and 90 °C.

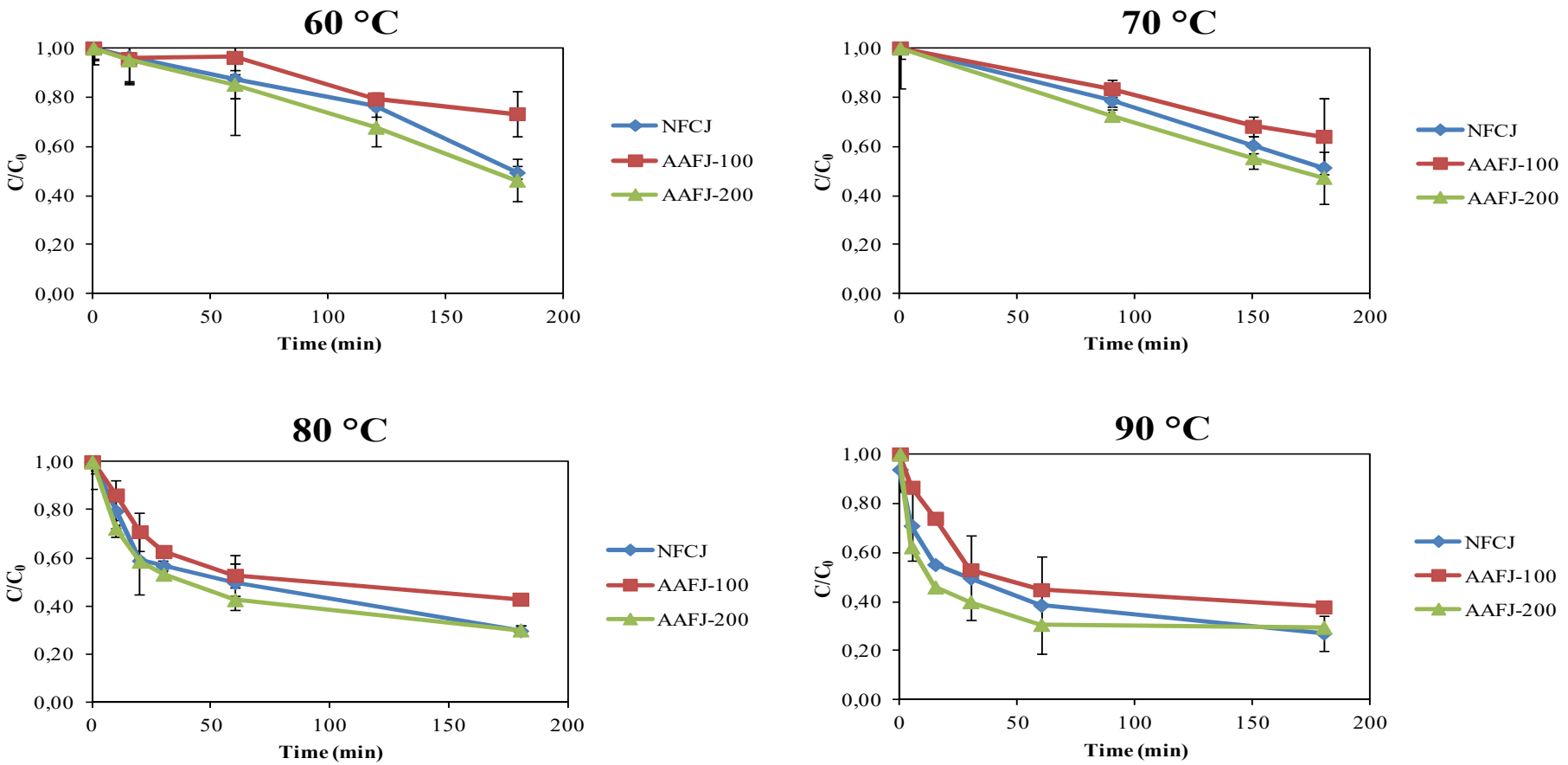


Figure 21: Effect of ascorbic acid fortification (AAFJ-100, AAFJ-200) on anthocyanins kinetic degradation during thermal treatment at temperature 60, 70, 80 and 90 °C

IV- Analysis of prepared steamed yoghurt

IV-1- Physico-chemical analysis of yoghurt

Physico-chemical properties of the manufactured steamed yoghurts (standard yoghurt, yoghurt with non fortified pomegranate juice, yoghurt with pasteurized pomegranate juice at 60 °C/15 min and yoghurt with commercial pomegranate juice) were shown in table 09.

Table 09: Physicochemical analysis of steamed prepared yoghurts.

	pH	Acidity (°D)	°Brix	Viscosity (g)	Total dry extract (%)	Fat content (%)	Protein content (%)
Standard yoghurt	*4.55*	75	7.70	28.93	18.81	3	3.28
Yogthurt with NFCJ	4.9	80	8.32	18.31	18.54	2.85	3.93
yoghurt with P- NFCJ	4.61	80	7.50	18.30	18.17	2.75	2.85
Yoghurt with CPJ	4.65	85	9.60	21.57	18.62	2.9	3.02
Norms	4.4-5.7	75-100			23.9- 25.15	2.75-3.15	2.85-3.15

NFPJ: non fortified pomegranate juice, P-NFPJ: pasteurized non fortified pomegranate juice at 60°C/15min, CPJ: commercial pomegranate juice.

- Results of this analysis revealed that pH, soluble solids content, acidity, fat and protein content determination were conform to norms. However a decrease in total dry extract and viscosity were observed after addition of pomegranate juices to standard yoghurt. This may be related to juices impact on the aggregation of casein network in yoghurts via electrostatic interaction, and on the resistance for the yoghurt matrix to flow. Indeed the addition of plant extracts generally decreased the consistency of the products owning to reduced water-binding capacity of proteins **(El-Said *et al.*, 2014)**

IV-2- Microbiological analysis

Microbial quality of the manufactured steamed yoghurts was given in table 10.

Table 10: Microbiological analysis of formulated yoghurts

	Total coliforms at 37°C	Yeasts and moulds	*Enterobacteria at 37°C*	*Streptococcus thermophilus*	*Lactobacillus bulgaricus*
Standard yoghurt	*Absent*	Absent	Absent	1.6×10^8	1.2×10^5
Yoghurt with NFPJ	Absent	Absent	Absent	1.7×10^8	1.3×10^5
Yoghurt with P-NFPJ	Absent	Absent	Absent	1.5×10^8	1.3×10^5
Yoghurt with CPJ	Absent	U	5.00 + 0.1	1.7×10^8	1.4×10^5
Norms	<10	Absent	Absent	$\geq 10^8$	$\geq 10^5$

NFPJ: non fortified pomegranate juice, P-NFPJ: pasteurized non fortified pomegranate juice at 60 °C/15min, CPJ: commercial pomegranate juice, U: uncountable

- Moulds, yeast and coliforms, are the primary contaminants in yoghurt (**Amakoromo *etal.,* 2012**), were not detected in yoghurt samples (NFPJ, P-NFPJ and standard). This illustrates the adequate heating treatment of milk, under strict aseptic conditions, during processing and manufacturing. However yoghurt flavored with CPJ was contaminated with very high levels of yeasts and moulds. It is possible that a high fungal contamination was in the commercial pomegranate juice. Pasteurization will rid juice of pathogens and other heat-sensitive microbes; therefore, it will reduce the microbial load substantially and extend the shelf-life of the product. If the original load is too high and/or the pasteurization process is inadequate or post-pasteurization contamination during cooling, bulk storage and bottling, some micro-organisms will survive. Indeed, yeasts and moulds were the most common organisms found in fruit juices. Some of micro-organism isolated from these products were capable of growing under refrigeration, completely spoiling the product before its expiration date **(Tournas *et al*., 2006**).

- Yoghurt enriched with pomegranate juice presented a slight increase in viability of lactic acid bacteria Flora (*Streptococcus thermophilus* and *Lactobacillus bulgaricus*) when compared with control yoghurt, which could be related with the composition of proliferation media (sugar and lipid) of samples after addition of pomegranate juice. The total viable numbers of lactic flora is an important parameter which contributes in the shelf life of yoghurt. This can be related to the

chemical composition of the pomegranate fruit; arils contain 85% water, 10% total sugars (mainly fructose and glucose), 1.5% pectin, and organic acids. **(Viuda-Martos *et al.*, 2010)**.

IV-3- Antioxidant activity and ANC content

It was noticed from table 11 that antioxidant activity of different steamed yoghurts measured by RSA revealed that the addition of pomegranate juice, increased significantly the inhibitory activity against DPPH˙ radical, compared with standard yoghurt (SY) prepared without *Punica granatum* juice, being pronounced in yoghurt with AAFPJ-100 (40.78 ± 0.51). The pomegranate juice contained total monomeric anthocyanin; these compounds were present and significantly highest in supplemented yoghurts, in the following order AAPJ-100 > NFPJ> P-NFPJ > CPJ > SY, but not in the SY sample, providing a confirmation of supplementation.

Table 11: Radical scavenging activity and total monomeric anthocyanins content of steamed prepared yoghurts

	Radical scavenging activity (%)	**Total monomeric anthocyanin (mg/L)**
Standard yoghurt	15.83 ± 0.00^{e}	0.07 ± 0.06^{d}
Yoghurt with NFPJ	32 ± 0.00^{b}	4.27 ± 1.32^{a}
Yoghurt with AAFPJ-100	40.78 ± 0.51^{a}	4.93 ± 0.73^{a}
Yoghurt with NFPJ at 60°C/15min	25.00 ± 1.08^{d}	4.02 ± 0.03^{b}
Yoghurt with CPJ	31.71 ± 0.45^{c}	1.63 ± 0.62^{c}

Values with different letters (a-b-c-d-e) were significantly different (Tukey, $p < 0.05$) for the four types of yoghurt
NFPJ: non fortified pomegranate juice, AAFPJ-100: acid ascorbic fortified pomegranate juice (100ppm), CPJ: commercial pomegranate juice.

- Pomegrante juice is characterized by substantial amounts of phenolic compounds, including flavonoids namely anthocyanins **(Viuda-Martos *et al.*, 2010)**. . Therefore, ANC were well correlated and dominantly responsible for the antioxidant activity. The pomegranate juice possess proton donating ability and in association with a number of hydroxyl groups in the ANC structures to stabilize free radicals it could due to their ability to quench hydroxyl radicals by transferring hydrogen atom to free radical. **(El-Said *et al.*, 2014)**. It is clear that, addition of

ascorbic acid (E 300) to PGJ gave the highest value, difference was statistically significant when $p < 0.05$, in the antiradical capacity, providing additional evidence of its antioxidant activity.

IV-4- Sensory analysis

The samples of different manufactured yoghurts with NFPJ, AAFPJ-100, CPJ, were sensory evaluated, and scores were recorded.

- **Design of experiment**

Designing an experiment is a fundamental step in order to verify if the collected data will be statistically valid (**Périnel et Pagès 2004**). In our study, an optimal plan was validated.

Design evaluation

A-Efficacity	1,000
D-Efficacity	1,000

- **Product characterization**

The figure 22 represents the characteristics ordered from the one having the highest discriminating power to the one that has the lowest discriminating power on the prepared steamed yoghurts.

As reported, the acid savor has the highest discriminating power, followed by consistency taste and color and finally the savor and sweet savor.

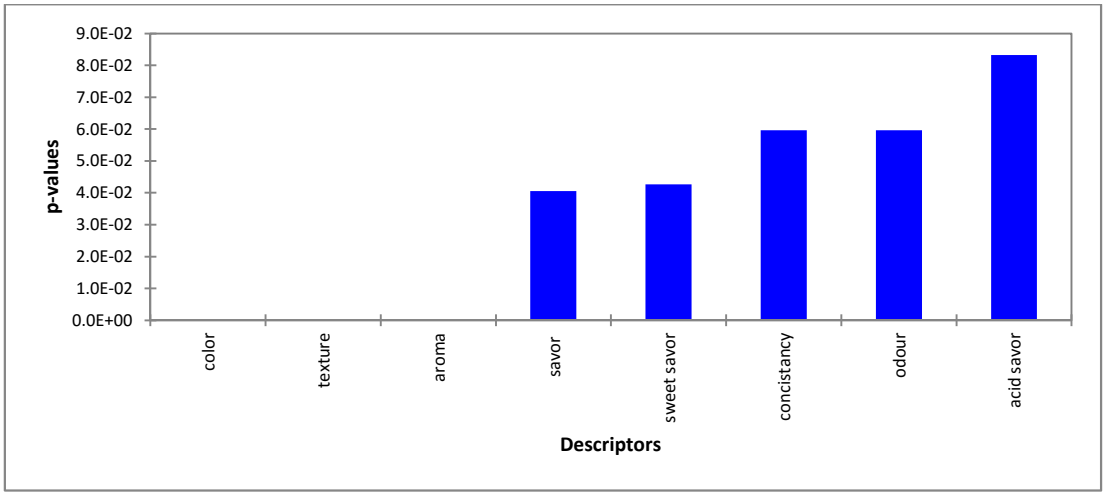


Figure 22: Discriminating power by descriptor.

- **Model coefficients**

The following graphics are very helpful to define our products. The blue color is associated to coefficient that has a significant positive value and the red color is associated to coefficient that has a significant negative value. White is associated insignificant value.

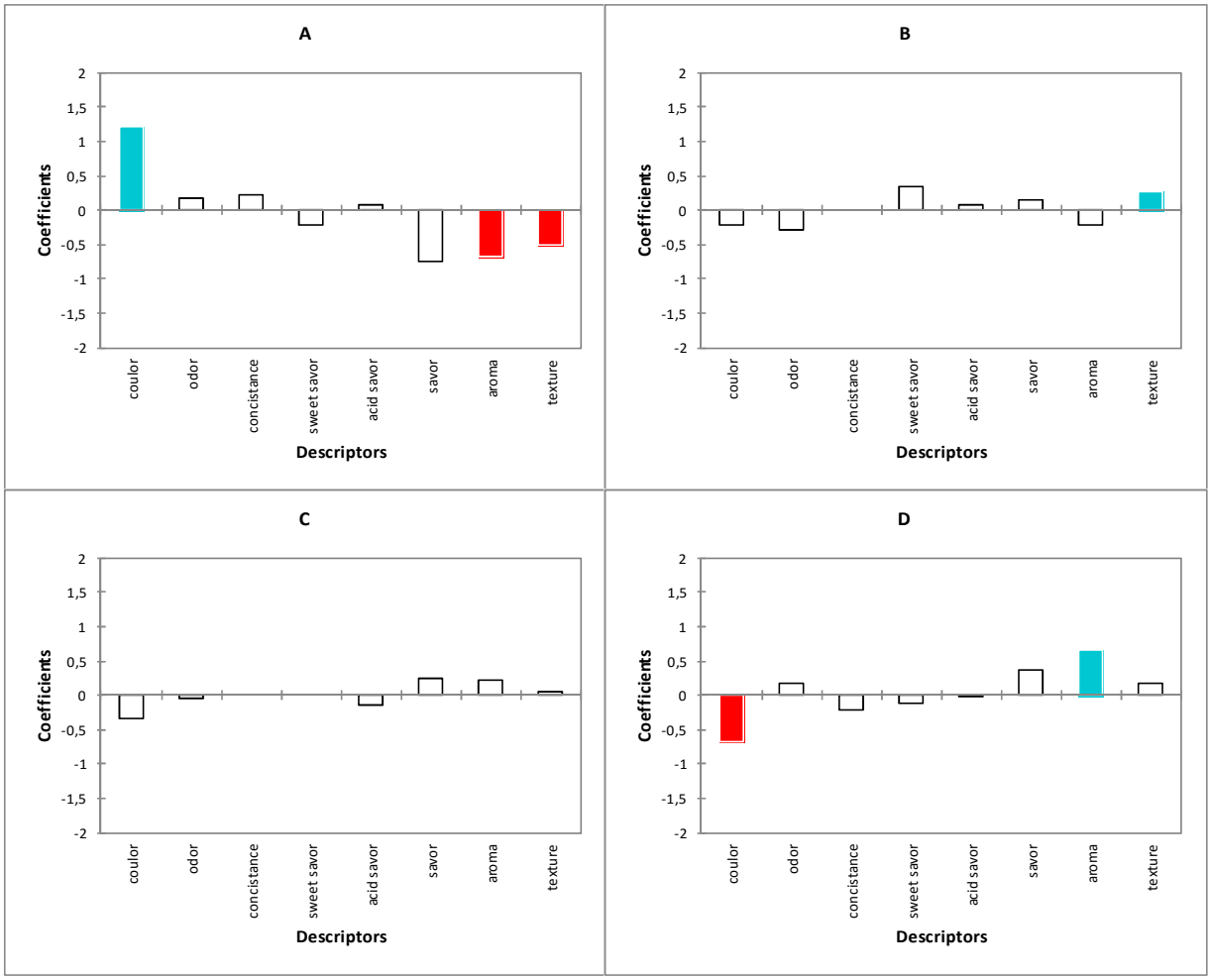


Figure 23: Model coefficients of yoghurts.

- **Preference**

The figure 24 represents the histograms of preference of each product by panelists. As it can be seen, the yogurt with commercial pomegranate juice was more appreciated than with fresh pomegranate juice. This supports the theory of adding flavor enhancer by industrial which does not change the taste but increases the intensity of the olfactory-gustatory perception (taste and / or smell) of the products.

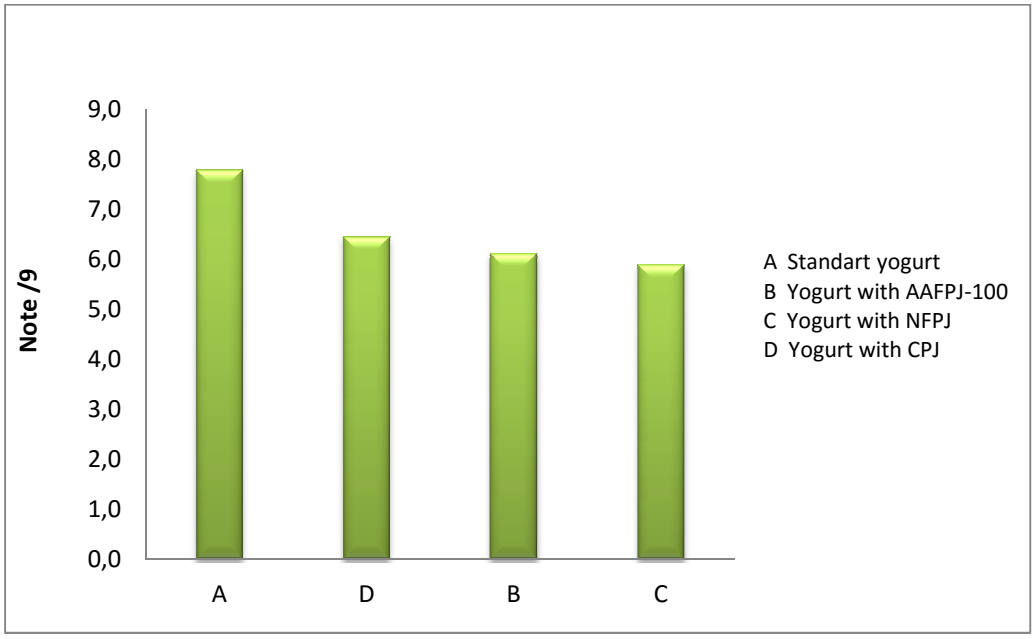


Figure 24: Preferences assigned to each product by panelist

Conclusion

Conclusion

The exposure of pomegranate juice (fresh and ascorbic acid fortified juice) to different time-temperature couples, reveals that anthocyanins and physicochemical parameters have varying behavior.

For heat-treated fresh juice, the physicochemical parameters showed a variability in some time-temperature couples, whereas anthocyanins presented a decrease in their concentrations at different heat treatments applied. It was noticed that these concentration tend to decrease at 80 °C and 90 °C.

For the same product, the effect of temperature on anthocyanins may vary according to the degree of freshness, product composition, the nature of anthocyanins, pH, Brix, humidity, light, … etc.

The fortification of pomegranate juice by ascorbic acid demonstrated that this later may have different impacts on the anthocyanins stability. The farmer results show that the anthocyanins degradation is accelerated by the presence of ascorbic acid at higher concentration (200 ppm).

This highlight how difficult is to isolate the effect of temperature and fortification with ascorbic acid to other physicochemical parameters but also how crucial is to master and optimize it in the food, pharmaceuticals and cosmetics processing, where anthocyanins are used.

Bio-yoghurt is a very favorite food which can be healthier by fortifying it with natural source of vitamins, antioxidant and phenolic compounds.

However, in the present work, only one variety is studied, in order to improve the understanding of anthocyanins degradation, it would be interesting to:

- Expand the study to other varieties;
- Vary the ascorbic acid fortification doses to better confirm its negative role on the anthocyanins of the studied variety and other varieties;
- Study the microbiological side of the pasteurized juice;
- Use the developed analytical techniques (HPLC, NMR ...) to characterize the compounds of degradation initially followed and understand the degradation mechanisms involved during thermal processing;

- Use pure natural juices in other dairy products for better safety and organoleptic characteristics.

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A

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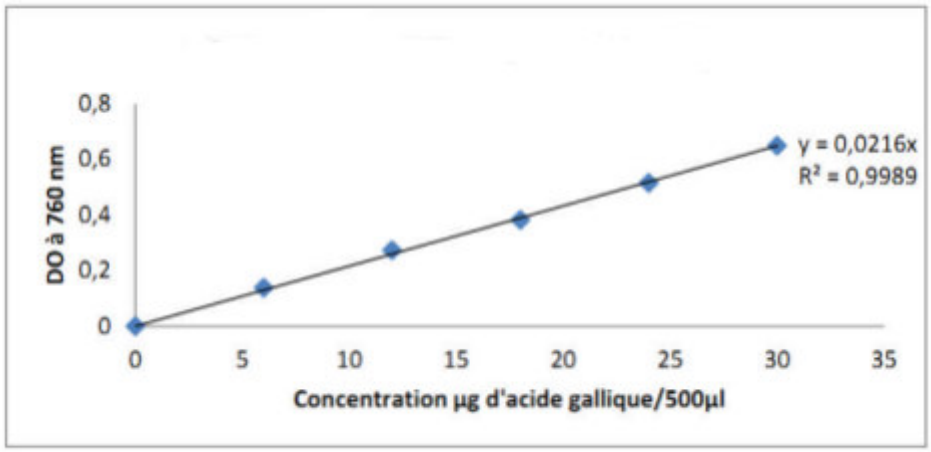
Appandix

Appendix 01

I- Calibration courbe of gallic acid



Courbe d'étalonnage d'acide galique

Appendix 02

Questionnaire d'évaluation sensorielle de quatre échantillons du yaourt

Sexe :F ou H, Profession……..

Date : 01juin 2016

Quatre échantillons de yaourt étuvé codés **A**, **B, C et D** vous sont présentés, il vous est demandé d'évaluer différentes caractéristiques et d'attribuer une note de 1 à 5 pour chaque échantillon sur l'échelle suivante :

1. Couleur *:*

1 : Pas appréciée

2 : Peu appréciée

3 : Moyennement appréciée

4 : Bien appréciée

5 : Très appréciée

Echantillon A	Echantillon B	Echantillon C	Echantillon D

Attribuez une note de 1à 9 pour chaque échantillon selon votre préférence par rapport à

La couleur :

A ☐ B ☐ C ☐ D ☐

2. Odeur :

1 : Très faiblement intense

2 : Faiblement intense

3 : Moyennement intense

4 : Fortement intense

5 : Très fortement intense

Echantillon A	Echantillon B	Echantillon C	Echantillon D

Attribuez une note de 1à 9 pour chaque échantillon selon votre préférence par rapport à

l'odeur :

A ☐ B ☐ C ☐ D ☐

3. Consistance :

1 : Trop liquide

2 : Liquide

3 : Faiblement mou

4 : Moyennement mou

5 : Mou

Echantillon A	Echantillon B	Echantillon C	Echantillon D

Attribuez une note de 1à 9 pour chaque échantillon selon votre préférence par rapport à

l'odeur :

A ☐ B ☐ C ☐ D ☐

4. Sensation en bouche :

A. Saveur

• Saveur sucré :

1 : Absent

2 : Faible

3 : Moyen

4 : Fort

5 : Très fort

Echantillon A	Echantillon B	Echantillon C	Echantillon D

Attribuez une note de 1à 9 pour chaque échantillon selon votre préférence par rapport à

la saveur sucrée :

A ☐ B ☐ C ☐ D ☐

 Saveur acide:

1 : Absente

2 : Faible

3 : Moyenne

4: Forte

5 : Très forte

Echantillon A	Echantillon B	Echantillon C	Echantillon D

Attribuez une note de 1à 9 pour chaque échantillon selon votre préférence par rapport à

la saveur acide :

A ☐ B ☐ C ☐ D ☐

 Attribution de la saveur

1. Aucune
2. fraise
3. fruits des bois
4. grenade

5. pèche

Echantillon A	Echantillon B	Echantillon C	Echantillon D

Attribuez une note de 1à 9 pour chaque échantillon selon votre préférence par rapport à

l'atribution de la saveur :

A ☐ B ☐ C ☐ D ☐

 Arôme : (note)

1 : Absent

2 : Faible

3 : Moyen

4 : Fort

5 : Très fort

Echantillon A	Echantillon B	Echantillon C	Echantillon D

Attribuez une note de 1à 9 pour chaque échantillon selon votre préférence par rapport à

l'arome :

A ☐ B ☐ C ☐ D ☐

B. Texture

1 : Très lisse

2 : : Lisse

3 : Moyenne

4 :Granuleuse

5 : Très granuleuse

Echantillon A	Echantillon B	Echantillon C	Echantillon D

Attribuez une note de 1à 9 pour chaque échantillon selon votre préférence par rapport à

la texture :

A ☐ B ☐ C ☐ D ☐

5. Classez selon d'ordre de préférence les échantillons (A, B, C ou D) en leur attribuant une note de 1 à 9 :

	Echantillon A	Echantillon B	Echantillon C	Echantillon D
Classenment				
Note				

6. Quels sont les caractéristiques qui ont motivé votre préférence ?

1 : La couleur

2 : L'odeur

3 : La texture

4 : Le gout

5 : La consistance

Autre…….....

*** Merci pour votre coopération ***