Department of Agricultural, Food and Environmental Science
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EVALUATION OF FOLATES CONTENT IN BERRY SPECIES CULTIVATED IN DIFFERENT EUROPEAN COUNTRIES.

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DEDICATION

To all my entire Family

Table of Contents

SUMMARY

The good berry project involves many partners and is aim at improving the stability of high-quality traits of berry in different environments and cultivation systems for the benefit of European farmers and consumers.

However, in few decades there has been an increase interest in studies concerning all kinds of fruits. Particularly berry fruits, which are well studied as they contain the best dietary sources of bioactive compounds such as folic acid. This molecule is usually used as a functional food ingredient and in food fortification and helps in the prevention of many diseases and is also very important vitamin to the growth of the foetus's spinal cord and brain.

A study was conducted to evaluate the folate or Vitamin B9 content of strawberry, raspberry and black currant cultivars (WP1) and of 126 strawberry selections (F1 generation) resulting from a cross between two varieties, Senga Sengana and Candonga (WP3), cultivated in different European environments. The evaluation was done by chromatography technique of HPLC.

In WP1 the highest folate levels among the 3 cultivars was seen in strawberry, cultivar 1_A (84.58 ug/100g FW) cultivated by CIREF in France. The second was raspberry, cultivar 4_B (65.84 ug/100g FW), still cultivated by CIREF in France. The last fruit folate levels were seen in cultivar B_11 of the black currant fruit cultivated by NIBIO in Norway.

In WP3 the highest folate levels (123.40 ug/100g FW), were seen in fruits H027 selection cultivated by HANSABRED in Germany.

LIST OF TABLES

LIST OF FIGURES

LIST OF PICTURES

1. INTRODUCTION

1.1 Background

The good berry (strawberry, raspberry and black currant) project is a multidisciplinary, collaborative project based on complementary expertise and skills of internationally recognized berry research institutions and involves some key berry small and medium size enterprises that will combine their effort to secure the robustness of the results, using different cultivation techniques to molecular studies. This project involves many partners or research institutions found in the following countries; Spain, Germany, Norway, Italy, Poland, France, United Kingdom, Belgium, Chile, and China. Each of the partners involved in this project have a well-defined role, detailed in a work plan or work package. The work description of Universita Politecnica Delle Marche (UPM), which is one of the beneficiaries of this project, is defined within work packages 1 and 3 (WP1 and WP3). WP1 is aim at evaluating the effect of different environment on fruit quality traits of well establish strawberry, raspberry and black currant cultivars, while WP3 focuses on the evaluation of quality traits 126 strawberry selection (F1 generation), originating from Senga Sengana and Candonga.

In line with the above, the Good berry project is aim at improving the stability of high-quality traits of berry in different environments and cultivation systems for the benefit of European farmers and consumers. However in order to improve stability of high quality traits, the selection of model species can be considered as strategic and important using most recent technical advances such as: the identification of berry germplasm exhibiting advantageous balance of production versus nutritional quality throughout the European Union, the search of innovative production systems to maintain high yield in a range of European-wide environments, and the development of standardized and reliable analytical tools to evaluate berry production and fruit quality.

However, in the last few decades there has been a constant increase of popularity and an interest regarding research of all kinds of fruits. Particularly fruit berries, which are well studied as they contain the best dietary sources of bioactive compounds (BAC) (De Souza *et al*., 2014). These bioactive compounds are abundant especially in highly-colored berries. The species that contain most BAC belong to members of several families, such as Rosaceae (strawberry, raspberry,

blackberry), and Grossulariaceae (black currant). They are globally known and consumed, and these berries' BAC such as folic acid is used as a functional food ingredient and in food fortification. Berries, especially straw berry are among the most important crops in Europe and the production of raspberry and blackcurrant are increasing strongly in recent years. Berries are important among fruits because of the presence of bioactive folate or vitamin B9. Folic acid is the synthetic form of folate, a water-soluble vitamin also known as vitamin B9. Folic acid (FA) stands out as a molecule having biological importance in recent years. The name folate usually outlines a class of compounds with chemical structures related to pteroylmonoglutamic acid and generally recognised as folic acid (FA, vitamin M, B9, (Deconinck *et al*, 2011).).

Since FA is more stable, cheap and easily absorbable in its synthetic form, it reveals its higher bioavailability than naturally occurring food folate. This water-soluble B group vitamin is increasingly utilized for food fortification purposes (Hau et *al*, 2009). Also, folic acid is an essential compound involved in many important biochemical processes in humans, mainly in its ionic form. It is involved in cell multiplication, regulation of gene activity, red and white cell production, renewal of skin and the intestine lining, as well as in the synthesis of chemicals that modulate brain function. At physiological pH the acid-base form is folate. Very often, the term folic acid refers to the fully oxidized synthetic compound used in dietary supplements, whereas folate refers to the various tetrahydrofolate derivatives naturally present in foods. Nevertheless, there is no difference between natural and synthetic folic acid. It is the same molecule and with the same effects (Marchetti *et al*., 2014).

The recommend daily intakes (RDI) for folate change from 150 to 600 µg per day depending on the age and sex of the individuals and vary notably from country to country (Fajardo *et al*, 2012). The United States Public Health Service suggests that all women of childbearing age should use 400 µg daily dose of folic acid for the prevention of spina bifida or other neural tube defects. Currently, some European countries recommend that intake 500 µg of daily folic acid for women who are breastfeeding (Fajardo *et al*., 2012).

As a consequence of this, this research work is focused on the evaluation folate content of some well-known established cultivars, of strawberry, raspberry and blackcurrant, with diverse adaptability to different environments through a

comparative study and the evaluation of folate content of 120 strawberries individuals (F1 generation) resulting from a cross or breeding between two varieties, Senga Sengana and Candonga, which are adapted to different areas (Northern and Southern Europe) , for the benefit of European farmers.

1.2. Literature review -

1.2.1 Categories of the good berry

1.2.2 Strawberry

Strawberries (family: *Rosaceae, genus Fragaria, cultivated: Fragaria* × *ananassa*, wild: *F. virginiana*) belong to berries that are popular due to their desirable sweet taste and attractive aroma, with smooth texture and red color. The plant is acclimatized to different environments and, therefore, could be cultivated worldwide, intensively in Europe and North America in open fields, whereas in China it is cultivated mainly in greenhouses (Wang *et a*l., 2015).

There were more than 600,000 acres and 3.9 million tons of strawberries produced worldwide in 2005. The next largest production regions for strawberries are the Russian Federation, and USA, which produced 1.1 million tons of strawberries (Strik *et al*., 2007). Amongst the fruits, fresh strawberries are considered to have the highest content of vitamin c. Biocative copmounds in strawberries also help to lessen the risk of cardiovascular incidents by inhibition of LDL-cholesterol oxidation or improved vascular endothelial function. This could reduce the risk of incidence of thrombosis (Basu *et al*., 2010) (Prasath *et al*., 2014), It is known that some compounds present in strawberries, such as ellagic acid and quercetin (Edderkaoui *et al*., 2013) have demonstrated anti-cancer activity in their purified forms or fractions, sometimes enriched with specific components. The preventative effect of berry fruits for human esophageal cancer is because of their potential to modify exposure of several genes relating to the progress of oral cancer (Chen *et al*., 2012).

The protection from tumorigenesis upon pre-treatment with strawberry extracts was observed for breast cancer in mice too, but the mechanism by which it exerts the chemoprevention is still not clear. Protective effects of strawberry extracts on human dermal fibroblasts was also referred (Giampieri et al., 2014) to (Wiseman *et al*. 2002), strawberry (*Fragaria* × *ananassa*) is a relevant source of bioactive compounds because of its high levels of vitamin C, folate, and phenolic constituents.

According to the available data (Tulipani *et al*., 2008), the intake of dietary folate through strawberry consumption is interesting. For example, 250 g of strawberries (w60 mg of folate on average) can supply 30% of the daily European and U.S. folate recommended daily allowances. Moreover, the strawberry, although to a lesser extent, is a source of several other vitamins, such as thiamin, riboflavin, niacin, vitamin B6, vitamin K, vitamin A and vitamin E Moreover, strawberries are economically and commercially important and widely consumed fresh or in processed forms, such as jams, juices, and jellies. That is why they are among the most studied berries from the agronomic, genomic, and nutritional points of view. The straw berry fruits can be seen in **picture 1**.

Picture 1: A view of strawberry fruits

1.2.3 Raspberry

The raspberry is the edible fruit of a multitude of plant species in the genus *Rubus* of the family *Rosaceae*, most of which are in the subgenus Idaeobatus; the name also applies to these plants themselves. Raspberries are perennial with woody stems. Raspberry fruits contain iron, vitamin C, and antioxidants and are usually eaten fresh, often with cream or ice cream, as a dessert fruit. Several different sub-species of raspberries exist. However, the most popular commercial cultivar in practice is red-raspberry, which is the result of hybridization between *R. idaeus* (European raspberry) and *R. strigosus* (American raspberry) types. Red raspberyry belong to the red-colored Rubus fruit cultivars grown in Europe (European red raspberry), North America (American variety), and many different cultivars and varieties in Asia, that *is R. hirsute's* growing in China (Fu *et al*., 2015). Red raspberries are the fourth most significant fruit product in the world. The similarly planted areas of red raspberries include Europe and Asia. In 2005 Europe (Serbia and Montenegro,

Poland) produced 231,000 tons, Asia (Russian Federation, mainly), 131,000 tons, while North America produced about 16% of the red raspberry tonnage in the world, in the USA (Wang *et al*., 2015)

Raspberries are called bramble fruit and are an aggregate of drupelets. They have a very popular attractive flavor (taste and aroma) for consumers. They are also great source of vitamins such as ascorbic acid. According to (Benvenuti *et al*., 2004) among the berry species, raspberries have similar content to strawberries and blackberries, about three-times more ascorbate than blueberries have, but less than in red currants, and several times less than black currant vitamin content.

The fruits have been used in traditional and alternative medicine for a long time to cure wounds, colic, diarrhea, and renal illnesses (Zhang *et a*l., 2011). Also, Raspberries could also be helpful in the diet targeted for managing early stages of type II diabetes and hypertension (Cheplick *et al*., 2007). Similarly, Raspberry extracts, some individual polyphenols (anthocyanins, ellagitannins, and ellagic acid); (McDougall *et al*., 2008). Finally, according to (Wedge et al., 2011), Raspberry extracts have shown anti-proliferative effects to suppress the growth of human colon, prostate, breast, and oral tumor cells and the effect is comparable with other common berry extracts. The simple view of the raspberry fruit attached to the plant can be seen in **picture 2**.

Picture 2: Raspberry fruits

1.2.4 Black currant

Black currants (*Ribes nigrum L*.), a native species to central and northern Europe as well as northern Asia are considered a rich source of vitamin C. Black currants are particularly popular in Europe, where most of the fruits are consumed as processed forms (juices, purees, syrups, jams, jellies) and only a small portion as fresh

products. Black currant like fruit and vegetable play an important role in human nutrition and health, particularly as sources of vitamin C, thiamine, niacin, pyridoxine, folic acid, minerals and dietary fibre (Wargovich 2000). The fruits of black currants provide an inexhaustible source of vitamins which along with minerals makes the fruit highly physiologically valuable. In addition to high vitamin C content, black currants contain a high level of bioactive compounds with potential health-promoting properties. A recent study shows that black currant is a very rich source of phenolic compounds and among the 143-vegetable food analysed, black currant was included in the top 10 list in terms of their polyphenol concentration (Ovaskainen *et al*., 2008). A bunch of black currant fruits can be seen in **picture 3.**

Picture 3: A bunch of Black currant fruits

1.3 Folic acid/ vitamin B9

Folate is a water-soluble vitamin, also known as vitamin B9 and the name folate usually outlines a class of compounds with chemical structures related to pteroylmonoglutamic acid and generally recognised as folic acid (FA, vitamin M, B9 or B11); (Deconinck *et al*, 2011). Since FA is more stable, cheap and easily absorbable, it reveals higher bioavailability than naturally occurring food folate, this water-soluble B group vitamin is increasingly utilized for food fortification purposes (Hau et al, 2009). The recommend daily intakes (RDI) for folate change from 150 to 600 µg per day depending on the age and sex of the individuals and vary notably from country to country (Fajardo *et al*, 2012). Similarly, the United States Public Health Service suggests that all women of childbearing age should use 400 µg daily dose of folic acid for the prevention of spina bifida or other neural tube defects. Currently, some European countries recommend that intake 500 µg of daily folic acid for women who are breastfeeding as indicated by (Fajardo *et al*., 2012).

1.4 The formula and chemical structure of folic acid

The chemical formula of folic acid is $C_{19}H_{19}N_7O_6$-and its molar mass is 441.40 g mol-The molecule is formed by a long chain of 6 members, which also contains one aryl ring join to a dihydropteridine ring. Its chemical structure can be written as seen in **figure 1.**

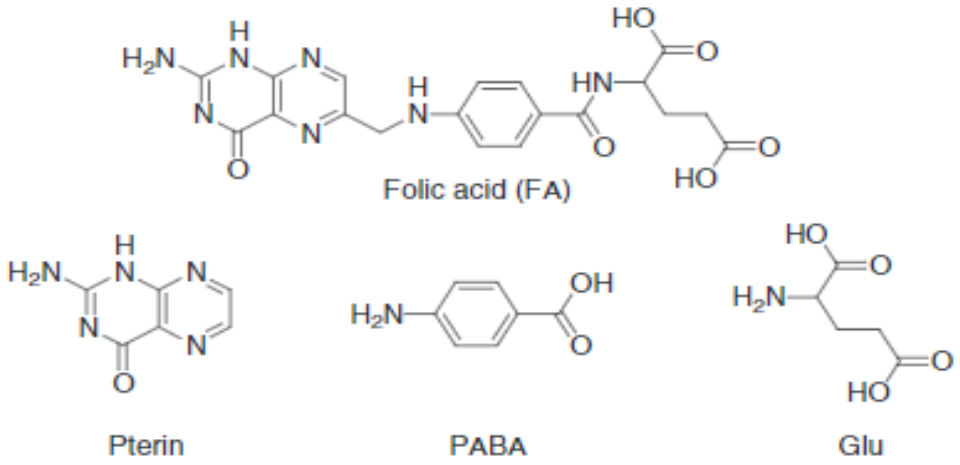

Figure 1. Molecular structure of folic acid and the different moieties; pterin, para-aminobenzoic acid (PABA) and glutamic acid (Glu) that form a folic acid molecule: (Adams, 1995; Santos and Pereira, 2007).

1.5 Biochemistry of folate

Folate is a generic term used for a group of compounds with a basic structure consisting of a pterine linked through a methylene bridge to p-aminobenzoic acid to which one or more glutamate residues are attached by γ-peptide bonds. The pterine moiety exists in three oxidation states (oxidised, partially reduced as 7,8-dihydrofolate and fully reduced as 5,6,7,8-tetrahydrofolate) and can be substituted at the N-5 or N-10 position by different one-carbon units (Gregory, 1989). Tetrahydrofolate (THF), which is the fully reduced form of the vitamin, carries one-carbon units at one of three different oxidation levels ranging from methanol to formate.

In the cell, five different one-carbon substituted forms of THF are present: 10-formyl-THF; 5-formyl-THF; 5,10-methenyl-THF; 5,10-methylene-THF; and 5-methyl-THF; each of these forms is interconverted in the cell through enzyme-

mediated catalysis. In the body, addition of glutamate residues to the monoglutamate form increases the affinity of folate cofactors for folate-dependent enzymes and is required to retain folates within the cell and subcellular organelles.

Naturally occurring food folates are reduced vitamers which are usually polyglutamates containing five to seven glutamate residues. Natural folates are unstable, and some losses occur in the presence of light, oxygen and at high temperatures. In contrast, the synthetic form of the vitamin, folic acid, is a fully oxidised monoglutamate and is the most chemically stable form. However, folic acid is not a natural component of the diet and is consumed only via fortified foods or food supplements (Brody, 1991). It has vitamin activity after having been fully reduced.

1.6 Sources of folic acid

Although the terms folic acid and folate are used interchangeably, the metabolic effects can be slightly different. Folic acid which is found in supplements and fortified food is the synthetic form of folate. Folate is found naturally, mainly in plants (US National Institutes of Health; 2014). Folate is found in plants and vegetables such as dark leafy greens, broccoli, asparagus, citrus fruits (oranges, grapefruits, strawberries), beans, avocado, peas and lentils, okra, Brussels sprouts, nuts and seeds, cauliflowers, beets, corn, celery, carrots and squash as dispulated by Deconinck *et al*., (2011) and Hau *et al*., (2009). Folate can also be found in meat product including chicken, turkey, lamb, beef and pork liver. Folic acid, on the other hand, can be found in fortified foods, such as cereal, pasta, flour, grains and bread. Folic acid supplements are sold over the counter in tablet or powder forms. The daily recommended allowance (RDA) of folic acid in the United States is 400 mcg/day for teenagers and adults, 500 mcg/day for breast-feeding women and 600 mcg/day in pregnancy Fajardo *et al*., (2012).

The FDA developed Daily Value (DV) to help consumers compare the nutrient contents of products within the context of a total diet. The DV for folate used for the values in Table 2 is 400 mcg for adults and children age 4 years and older (U.S. Food and Drug Administration, 2013). This recommended daily value however, is changing to 400 mcg DFE (dietary folate equivalent, also known as the amount of folate that the body can absorbed) as the updated Nutrition and Supplement Facts

labels are implemented. Manufacturers will use the following conversion factors: 1 mcg DFE = 1 micrograms (mcg) naturally occurring folate = 0.6 mcg folic acid.

1.7. The function of folic acid metabolism

Folates is a group of heterocyclic compounds based on a 4-[(pteridin-6-ylmethyl) amino] benzoic acid skeleton conjugated with one or more L-glutamate units. Over 150 folate forms exist in theory, but in most animals and plants less than 50 are found. In nature, folates exist in reduced form but during sample preparation and storage folates may be partly or totally oxidised. Causes of structural variations are: Oxidation state of the pterin ring, Number of glutamate (Glu) residues, often 3-11, One-carbon substituents at N-5 and/or N-10.

1.7.1 Folate absorption

Folate absorption is optimal at pH 6.3 Russell *et al*., (1979). Zhao et al., (2009) stated that passive diffusion across the cell-membrane is limited. Qiu et al., (2006) indicated transport across the luminal membrane is by highly specific transporters, mainly the protein-coupled folate transporter (PCFT), which helps in mediating the intestinal absorption of folate - (Vitamin B9), and its delivery to the central nervous system. Also, Assaraf., (2006) indicated that the absorption of folate occurs through passive transport across the basolateral membrane into plasma by less specific transporters. In addition, affinities vary between folate forms, being higher to reduced folates than folic acid for both PCFT and reduced folate carrier (RFC), which is a bidirectional anion transporter, the major uptake route of reduced folates, essential for a spectrum of biochemical reactions (Zhao et al., 2009). Folate absorption may also occur in the colon (Aufreiter et al., 2009). Similarly, Aufreiter et al., (2009) also indicated that although colon absorption rate has been found to be ~50 times lower than estimated in the small intestine, it may contribute significantly to total folate absorption since the transit time in the colon is longer (~15 times) and due to the presence of microbial folate production.

1.7.2. Distribution and physiological function of folates

After absorption, folates are transported to the liver, which is estimated to comprise 50% of the total folate body pool (Gregory et al., 1998). According to Lin *et al*., (2004), reabsorption of folates from bile in the gastrointestinal tract is suggested to

blunt-between meal fluctuations in folate supply to cells. However, estimations of folate content in bile vary greatly from 227 nmol/d to 5300 nmol/d (Lin *et al*., 2004). Therefore, the estimated size of the total human body pool of folate varies from 17 to 225 mmol (Gregory et al., 2001). In the cells, folate polyglutamates are retained and used in the nucleus as substrates in both nucleotide synthesis and the methylation cycle (Figure 2**)**. H4 folate-polyglutamates are required for pyrimidine and purine synthesis, and thus DNA and RNA production, by providing one-carbon atoms.

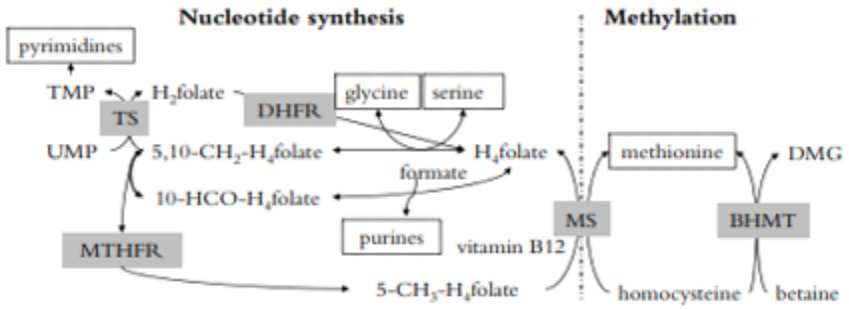

Figure 2: Folate involvement in nucleotide synthesis and the methylation cycle (Reed *et al*., 2006). Betaine-homocysteine methytransferases (BHMT): Dihydrofolate reductase (DHFR): Dimethyglycine (DMG): Methionine synthase (MS): Methylenetetrahydrofolate reductase: (MTHFR): Thymidine momophosphate(TMP): Thymidylate synthase (TS): Uridine monophosphate (UMP).

1.7.3 Excretion of folate

About 0.3-0.8% of the folate body pool has been estimated to be excreted intact or as catabolites daily (Gregory *et al*., 2001). Similarly, excretion of intact folate with urine is as low as ~5% of ingested folates (Lin et al., 2004; Witthöft et al., 2003) due to folates being efficiently reabsorbed from primary urine by the kifney. Studies shows that, in human faeces, folate content is about 400 nmol/day (Lin *et al*., 2004; Gregory & Quinlivan, 2002). Folates found in faeces originate from bacterial production, lysed enterocytes and gastrointestinal secretions such as bile (Gregory & Quinlivan, 2002). The 400 nmol folate excreted with faeces and stomal effluents daily contains about 20% non-absorbed food folates (Witthöft *et al*., 2006; Lin *et al*., 2004; Konings *et al*., 2002), which indicates incomplete bioavailability of folates.

1.8 Bioavailability of Folic acids

The biological availability of folates in foods has been difficult to assess quantitatively. When formulating nutrition recommendations, a food folate bioavailability of 50% of that of folic acid is commonly used (CDC, 2009; NNR, 2005). One of the most cited references for this estimate is a 92-day trial assessing the minimum food folate intake required for normalisation of folate status after depletion (Sauberlich *et al*., 1987). Although the size of doses of food folate (n=3) and folic acid (n=4) varied greatly in that trial, it was estimated that availability of food folates was 50% of that of synthetic folic acid consumed with food (Sauberlich *et al*., 1987).

The bioavailability of folates from various foods is dependent on several factors, including: intestinal deconjugation of polyglutamyl folates, the food matrix, the instability of certain labile folates during digestion (or before ingestion), the presence of certain dietary constituents that may enhance folate stability during digestion (e.g. folate-binding proteins, ascorbate). According to Melse-Boonstra *et al.* (2002), approximately two-thirds of total folate intake from a mixed unfortified diet is in the polyglutamyl form, derived mainly from vegetables. These polyglutamates need to be hydrolysed to the monoglutamate form for normal absorption in the proximal small intestine. This process is controlled by the intestinal brush-border enzyme glutamate carboxypeptidase II (GCPII). Also, since folates are covalently bound to macromolecules in foods, the food matrix and its components can also influence folate bioavailability by entrapment in the matrix, thereby hindering diffusion to the absorptive surface during digestion. According to Castenmiller *et al*., (2000), folate bioavailability from minced, chopped or enzymically-liquefied spinach is higher than that from whole spinach leaves when equal amounts of spinach folates are provided to human volunteers. Similarly, the effects of wheat bran and dietary fibre on folate bioavailability have been explored in various acute and longer-term studies in human subjects, but the results are inconsistent (Castenmiller *et al*., 2000).

Table 1. Bioavailability to human of folates in foods

Food/feed stuff	Bioavailability in % reported range) and in ug/100FW
Banana	0 – 148
Cabbage	0 – 127
Eggs	35 – 137
Lima beans	0 – 181
Liver (goat)	9 – 135
Orange juice	29 – 40
Spinach	26 – 99
Tomatoes	24 – 71
Wheat germ	0 – 64
Brewers' yeast	10 – 100
Straw berry	74
Raspberry	46
Blackcurrant	17
Rose hips	96
Sea buckthorn	39
Cherry sweet	22
Blueberry	11

Source: Eur Food Res Technol (2003); Strålsjö *et al*. (2003), Tulipani *et al*. (2008)

1.9 Folate Intake and status

According to data from the 2013–2014 National Health and Nutrition Examination Survey (NHANES), most people in the United States consume adequate amounts of folate. Mean dietary intakes of folate from foods range from 417 to 547 mcg DFE per day for children aged 2–19 years. Average daily intakes of folate from food are 602 mcg DFE for males aged 20 and older and 455 mcg DFE for females.

Although most people consume adequate amounts of folate, certain groups, including women of childbearing age and non-Hispanic black women, are at risk of insufficient folate intakes. Even when intakes of folic acid from dietary supplements

are included, 19% of female adolescents aged 14 to 18 years and 17% of women aged 19 to 30 years do not meet the target (Bailey *et al*., 2003-2006). Similarly, 23% of non-Hispanic black women have inadequate total intakes, compared with 13% of non-Hispanic white women.

About 35% of adults and 28% of children aged 1 to 13 years in the United States use supplements containing folic acid (Bailey et al., 2003-2006) Measurements of erythrocyte folate levels also suggest that most people in the United States have adequate folate status. According to an analysis of NHANES 2003–2006 data, less than 0.5% of children aged 1 to 18 years have deficient erythrocyte folate concentrations (Yang *et al*., 2006). Mean concentrations in this age group range from 211 to 294 ng/mL depending on age, dietary habits, and supplement use. In adults, mean erythrocyte folate concentrations range from 216 to 398 ng/mL, also indicating adequate folate status (Yang *et al*., 2006).

2.0 Stability of folate

Most folates in foods and feedstuffs (that is, folates other than folic acid and 5-formyl-FH4) are easily oxidized and therefore are unstable to oxidation under aerobic conditions of storage and processing. Under such conditions (especially in the added presence of heat, light, and/ or metal ions), FH4 derivatives can readily be oxidized to the corresponding derivatives of dihydrofolic acid (FH2) (partially oxidized) or folic acid (fully oxidized), some of which can react further to yield physiologically inactive compounds. For example, the two predominant folates in fresh foods, 5-methyl-FH4 and 10-formyl-FH4, are converted to 5-methyl-5,6-FH2 and 10-formylfolic acid, respectively. For this reason, 5-methyl-5,6-FH2 has been found to account for about half of the folate in most prepared foods. Although it can be reduced to the FH4 form (by ascorbic acid), in the acidity of normal gastric juice it isomerizes to yield 5-methyl-5,8-FH2, which is completely inactive. It is of interest to note that, owing to their gastric anacidosis, this isomerization does not occur in pernicious anemia patients, who are thus able to utilize the partially oxidized form by absorbing it and subsequently activating it to 5-methyl-FH4. Because some folate derivatives of the latter type can support the growth responses of test microorganisms used to measure folates, and some information in the available literature may overestimate the biologically useful folate contents of foods and/ or feedstuffs. Substantial losses in the folate contents of food can occur as the

result of leaching in cooking water when boiling (losses of total folates of 22% for asparagus and 84% for cauliflower have been observed).

2.1 Folate Deficiency

According to Carmel *et al*., (2005), Isolated folate deficiency is uncommon; folate deficiency usually coexists with other nutrient deficiencies because of its strong association with poor diet, alcoholism, and malabsorptive disorders. Megaloblastic anaemia, which is characterized by large, abnormally nucleated erythrocytes, is the primary clinical sign of folate or vitamin B12 deficiency (Bailey *et al*., 2012). Its symptoms include weakness, fatigue, difficulty concentrating, irritability, headache, heart palpitations, and shortness of breath.

Folate deficiency can also produce soreness in and shallow ulcerations on the tongue and oral mucosa; changes in skin, hair, or fingernail pigmentation; gastrointestinal symptoms; and elevated blood concentrations of homocysteine (Stover *et al*., 2012).

2.2. Folic acid and diseases

There were many studies looking at the effects of folic acid and diseases. The diseases discussed in this review are mainly based on the reported studies and the meta-analyses. The summary of the reported studies and meta-analyses on the association of folic acid and diseases discussed here.

2.2.1. Hyperhomocysteinemia

Hyperhomocysteinemia or simply high plasma homocysteine level has been reported as a risk factor for atherosclerosis and coronary artery diseases (Stanger *et al*., 2004). Methylenetetrahydrofolate reductase (MTHFR) plays an important role as an enzyme in folate metabolism. The mutation of the MTHFR gene at position 677 CT, which converts alanine to valine, results in decreased enzyme activity. A significant reduction of 65% enzymatic activity in the homozygous MTHFR TT genotypes and 30% reduction in the heterozygous CT genotypes has been associated with elevated homocysteine levels, DNA hypomethylation and genomic instability. Homocysteine was postulated to cause atherogenesis and thrombogenesis leading to substantial fibrosis and muscle cell hyperplasia although the exact mechanism is still unknown (Welsch *et al*., 1997). Eight Observational studies also suggested that hyperhomocysteinemia is an independent risk factor for cardiovascular related

diseases. A maximal reduction in plasma homocysteine concentrations could be achieved with a minimal dose of 0.8 mg/day of folic acid (carlos *et al*., 2004).

2.2.2. Coronary artery disease

A meta-analysis by Qin *et al.,* (2012) indicated the effectiveness of folic acid supplementation in the reduction on cardiovascular disease (CVD) risk measured by the progression of carotid intima-media thickness (CIMT). A meta-analysis by Wang *et al*., (2012) suggested that coronary heart disease risk was inversely related to dietary folate supplementation and blood folate level. Similarly, a meta-analysis by de Bree *et al.,* (2007) suggested that the risk of cardiovascular disease was reduced with high folic acid supplementation due to the improvement on endothelial function. However, Miller *et al.,* (2010) commented that although folic acid was suggested from previous studies to have homocysteinelowering effect and might be beneficial in the prevention of CVD, folic acid on the contrary might also promote progression of atherosclerosis because it also stimulates cell proliferation. Subsequently, a meta-analysis by Miller *et al*., (2010) reported that folic acid supplementation did not have any effect on CVD or stroke. Likewise, Zhou *et al*., (2011) reported in their meta-analysis that folic acid supplementation had no effect on major cardiovascular events, stroke, myocardial infarction, acute coronary syndrome and vascular death. Finally, it was also reported that there is no reduction in the risk of cardiovascular diseases or mortality rate in those with history of vascular disease with folic acid supplementation. Therefore, it was recommended that trials with large sample sizes will be needed in the future, in order to answer this important clinical and public health question (Bazzano *et al*., 2006)

2.2.3 Stroke

According to Yang *et al*., (2012), there might be a potential benefit of stroke prevention with folic acid supplementation. Also, Wang *et al*., (2007) in a meta-analyses concluded that there was a significant reduction in the risk of stroke by 18% with folic acid supplementation and it was more beneficial in those; with more than 36 months of treatment, with a 20% or more reduction of homocysteine level, with minimal or no fortification of grain in their diet and with no history of stroke. It was therefore indicated in their findings that the risk of stroke was reduced with folic acid supplementation. Similarly, Huo *et al*., (2012) indicated that to prevent stroke,

folic acid supplementation was effective in statins consumption free populations and in those with partial or without folic acid fortification. Lee *at al*., (2010) suggested that the combination of folate and B vitamins in male patients has a potential benefit in the primary prevention of stroke.

2.2.4 Diabetes

Folic acid supplementation in patient with type 2 diabetes mellitus was seen to cause reduction in homocysteine levels and, therefore, contributed to better glycemic control (Title *et al*., 2006). Diabetes is associated with endothelial dysfunction due to the uncoupling of endothelial nitric oxide (NO) synthase enzyme. He also reported in a study on type 2 diabetes mellitus patients who were treated with folic acid (10mg/day for 2 weeks) versus placebo that folic acid supplementation improved endothelial dysfunction and was found to significantly improve fasting endothelium-dependent flow-mediated dilatation (FMD). Similarly, Xu *et al*., (2012) reported that supplementation with folic acid and vitamin B12 had the effect of protecting the capillaries of the kidney from damage, and the mechanism may have something to do with the effect of anti-oxygenation. On the contrary, Schneider *et al*., (2014) conducted a randomized trial and reported that high-dose folic acid treatment did not improve renal endothelial function and failed to reduce albuminuria in human subjects with diabetic nephropathy. Likewise, Fotiou *et al*., (2014) demonstrated that folic acid can protect diabetic rats against diabetic peripheral neuropathy and the reason may be related to the improvement of the expression of nerve growth factor levels.

2.2.5 Pregnancy issues

According to Imdad *et al*., (2011), during the pre-conception period, risks of stillbirths secondary to neural tube defects (NTDs) were reduced with folic acid supplementation by approximately 41%. It was suggested therefore, for public benefit and significant reduction of the risk of recurrent NTDs, that targeted folic acid counseling should be given to women with a previous pregnancy affected by NTDs (Grosse and Collins ,2007).

Also, several clinical intervention trials have tested the hypothesis that periconceptional supplemental folate can reduce NTD risk. One of these, a large, well-designed, multi-centered trial conducted by the British Medical Research

Council, found that a daily oral dose of 4 mg of folic acid reduced significantly the incidence of confirmed NTDs among the pregnancies of women at high risk for such disorders. Several subsequent studies **Table 2** have shown that periconceptional supplementation of folate can reduce the risk of NTDs. These have included trials conducted in the US, which found folate supplements (400–4,000 μg) effective in preventing NTDs in women with prior NTD pregnancies (Stevenson *et al*., 2000). While folate supplements do not appear to affect NTD case-fatality rates, reductions in NTD incidence are associated with reductions in neonatal deaths. A meta-analysis of eight observational studies indicated that folate supplementation was associated with a 46% reduction in NTD risk, which was associated with a 13% reduction in neonatal deaths (Blencowe *et al.,* 2010). A systematic review of 14 folate intervention trials pointed out that not all NTD cases can be prevented by folate; that analysis suggested that a rate of 8–10/10,000 live births or abortions would appear to involve factors not affected by increasing folate intakes (Heseker *et al*., 2009). However, there are many controversies on the continuous supplementation of folic acid during the second and third trimesters of pregnancy in the prevention of NTDs, unlike the well-recognized beneficial effects of its supplementation before and shortly after conception. Charles *et al*., (2005) found folic acid supplementation when given from time of initial antenatal appointment onwards to be of no benefit and no difference in birth and placental weight or gestational age of the pregnancy was observed, contrary to the Cochrane review, which reported that high doses of folic acid supplementation could reduce the risk of low birth weight. Similarly, Fekete *et al.,* (2012) reported no beneficial effect of folic acid supplementation on either the weight of the placenta or on gestational length. However, when doubling folate intake, there was an observed 2% increase in birth weight. Therefore, it was suggested that more research studying the effect of folate supplementation in pregnancy would be necessary and useful in order to develop further guidelines and recommendations for pregnant women (Lassi *et al.,* 2013).

Table 2: **Results of Placebo-Controlled, Clinical Intervention Trials of Folate Supplements in the Prevention of Neural Tube Defects**

Trial	Folate treatment	NTD Rates, Cases/Total pregnancies		PR (95% Cl)
		Placebo	Treatment	
1[a]	4mg	4/51	2/60	0.42 (0.04-2.97)
2[b]	4mg multivitamins	21/602	6/593	0.34 (0.10-0.74)
3[c]	0.8mg multivitamins	2/2104	0/2052	0.00(0.00-0.85)

Source: [a]Lawrence, K. M., James, N., Miller, M., and Campbell, H. (1980). Br. Med. J. 281, 1,542 (women with NTD histories). [b]Milunsky, A., Jick, H., Jick, S. S. et al. (1989). J. Am. Med. Assoc. 262, 2847 (women with NTD histories). [c]Czeizel, A. E. and Fritz, I. (1992). J. Am. Med. Assoc. 262, 1,634 (women without previous NTD births

2.2.6 Cancer

Low folate status has been associated with increased risk of cancers of the colon, cervix, lung, pancreas, prostate, mouth and pharynx, head and neck, stomach, and brain. Wilson *et al*., (1993), reported a study of women infected with human papilloma virus showed a five-fold increase in risk of cervical dysplasia when they also had low serum folates. Similarly, two large epidemiological studies have indicated that folate adequacy may reduce the effect of alcohol consumption in elevating breast cancer risk (Thomson *et al*., 2000). Meta-analyses of cohort studies have found food-folate intakes to be associated with reductions in colorectal cancer risk (Spiegelman *et al*., 2010). Studies in animal models have shown folate deprivation to promote colon carcinogenesis. However, a meta-analysis of five randomized clinical trials failed to find folate supplementation effective in reducing risk of recurrent colorectal cancers (Ibrahim *et al*., 2010).

These findings were rationalized with the fact that folate is required for cell proliferation, for which reason its deprivation would be expected to impair the tumor

growth. Thus, the question has been raised as to whether population-wide folate fortification may have cancer-promoting effects (Kim *et al*., 2004). In fact, the results of trials support that concern. The Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial, a prospective study involving 25,400 American women 55–74 years of age, found the incidence of breast cancer to be 20% greater for subjects reporting folate intakes of 400 µg/day compared to those with lower intakes. The Aspirin/Folate Polyp Prevention Trial, which involved 1,000 subjects with histories of colorectal adenomas randomized to 1 mg folic acid or a placebo, found after 2 years that folate treatment increased by 67% the risk of having a recurrent adenoma, and doubled the risk of having at least three adenomas (Cole *et a*l., 2007). However, it is clearly seen that, the role of folate in the biology of cancer is a complex one with a lot of controversial reports.

3. AIM OF STUDY

Generally, the importance of berries cultivation and consumption to human health can not be under estimated**.** In this light the aim of this work was to:

i. Evaluate by HPLC the amount of folate or Vitamin B9 content in strawberry, raspberry and black currant cultivars and of 126 strawberry selections (F1 generation) resulting from a cross between two varieties, Senga Sengana and Candonga, which are adapted to different areas (Northern and Southern Europe).

ii. Comparing the results of the folate content of the different cultivars (strawberry, raspberry and black currant) cultivated in different environments and the results of the strawberry selections also cultivated in the different environments in order to determine which cultivar and which strawberry selection best suit which environment for the benefit of European farmers.

4. MATERIALS AND METHOD

4.1 Plant Materials

Fruit samples analysed here were received in an already crushed state, from 10 different research institutions located in different EU countries, which are partners of the Horizon 2020- Good berry project. The variation of folate content was analysed in 33 samples of well-established cultivars of straw berry, raspberry and blackcurrant (WP1). In WP3, 126-strawberry selection (F1 generation, resulting from a cross between two varieties, Senga Sengana and Candonga, which are adapted to different area in Northern and Southern Europe,) all grown bypartners located in different cultivation areas in EU as seen in **tables 3 and 4** respectively.

Table 3: Materials for the evaluation of folate content in already established cultivars of strawberry, raspberry and black currant.

Research Institution	country	Short name	Number of samples	Code attributed to cultivar
Hochchule Geisenheim	HGU	Germany	2	HGU_18_R_(1-2-A)
Ciref creation varietale	Ciref	France	15	Ciref_S_(1-6-A and B)
				Ciref_18_R_(1, 4-A and B)
Norwegian Institute of bioeconomy research	NIBIO	Norway	16	NIBIO18_S,R,BC_(A and B)

Table 4: Biological materials for Straw berry selection (F1 generation)

Research Institution	country	Short name	Number of samples	Code attributed to cultivar
Universita Politecnica Delle Marche	Italy	UPM	91	UPM_H035-H0120
Andalusian Institute for Agricultural Research and Training in fisheries for food and organic production	Spain	IFAPA	35	IFAPA_18_H036-H085
Hansabred gmbh & co.kg	Germany	Hansabred	92	Hansa_H010-H121
Ciref Creation varietaleF fraises-fruits rouges	France	Ciref	105	Ciref_H001-H126

4.2 Extraction Procedure

Folates from (established cultivars and the selection of strawberry) were extracted according to the procedure described by Strålsjö *et al*. (2002), alongside some modifications. Homogenized berry samples (2 g) were extracted in duplicates for 12 min in a boiling water bath, in freshly prepared phosphate buffer (composed of 17.42g K_2HPO_4 and 13.62g of KH_2PO_4, 100ml distilled water, 1% (w/v) ascorbic acid, 0.1% 2-mercaptoethanol, PH 6.1 concentration 0.1M). After cooling, the samples were incubated with 1.5 ml hog kidney conjugase at pH 4.9 in a shaking water bath at 37°C for 3 h and the conjugase was then inactivated in a boiling water bath for 5 min. Subsequently, two centrifugations were carried out at (4500rpm, 4°C and 30min) and the supernatant collected and made to 25ml mark of the falcon tube, with the same phosphate buffer used for extraction. The final extraction was then stored at -20^{o}C until purification.

4.3 Solid Phase Extraction (SPE)

In order to separate and purify the folate from unwanted compounds, SPE technique was employed. The SPE cartridges were conditioned (activated and made more effective) using 2.5ml of methanol which was run twice through the cartridge and later equilibrated with same volume of water to ensure the interaction of the SPE material with the sample. Aliquots of samples were then loaded to the preconditioned cartridges and allowed to flow under vacuum through the SPE material. 0.7ml of eluting solution (0.1 mol/l sodium acetate containing 10% (w/v) sodium chloride, 1% (w/v) ascorbic acid 0.1% 2-mercaptoethanol), was then used to watch away unwanted materials from the cartridges and was discarded, and finally the second portion (3.5 ml) was collected and stored at -20^{o}C until further analysis.

4.4 High Performance Liquid Chromatography (HPLC) analyses

Quantification of folates by HPLC was carried out according to Stralsjo et al., (2003), whit some modifications. The HPLC system comprised a pump model PU-2089 (Jasco, Easton, MD, USA), a Fluorescence detector (FLD) FP-2020 Plus (Jasco, Easton, MD, USA) set at wavelengths of 290 nm excitation and 360 nm emission, and an autosampler AS-4050 (Jasco, Easton, MD, USA). The analytical column was a Luna C18, 250×4.6, 5 µm (Phenomenex, Torrance, California, USA). The mobile phase consisted of 30 mmol/l phosphate buffer, pH 2.3, using a gradient with acetonitrile starting at 6%, a lag time of 5 min and rising linearly to 25% within 20 min. The total run time was 33 min. Retention time of 10mins was used for peak identification, and quantification of folates content was determined through a calibration curve prepared by running standard concentrations of 5-methyl-tetrahydrofolic acid (5-CH3-H4folate). Results are expressed as µg 5-CH3-H4folate per 100g of fresh weight of strawberry (µg 5-CH3-H4folate/100g FW). All the samples were analysed in triplicate.

4.5 HPLC chromatograms

In order to accurate quantify and characterise the folate concentrations of all the berries, a calibration chromatogram obtained by running standard concentrations of 5-methyl-tetrahydrofolic acid (5-CH3-H4folate), with the retention time at which

folate content were determined was used as seen in **figure 3**. Folate concentration of the different berries were read by comparing them to the standard.

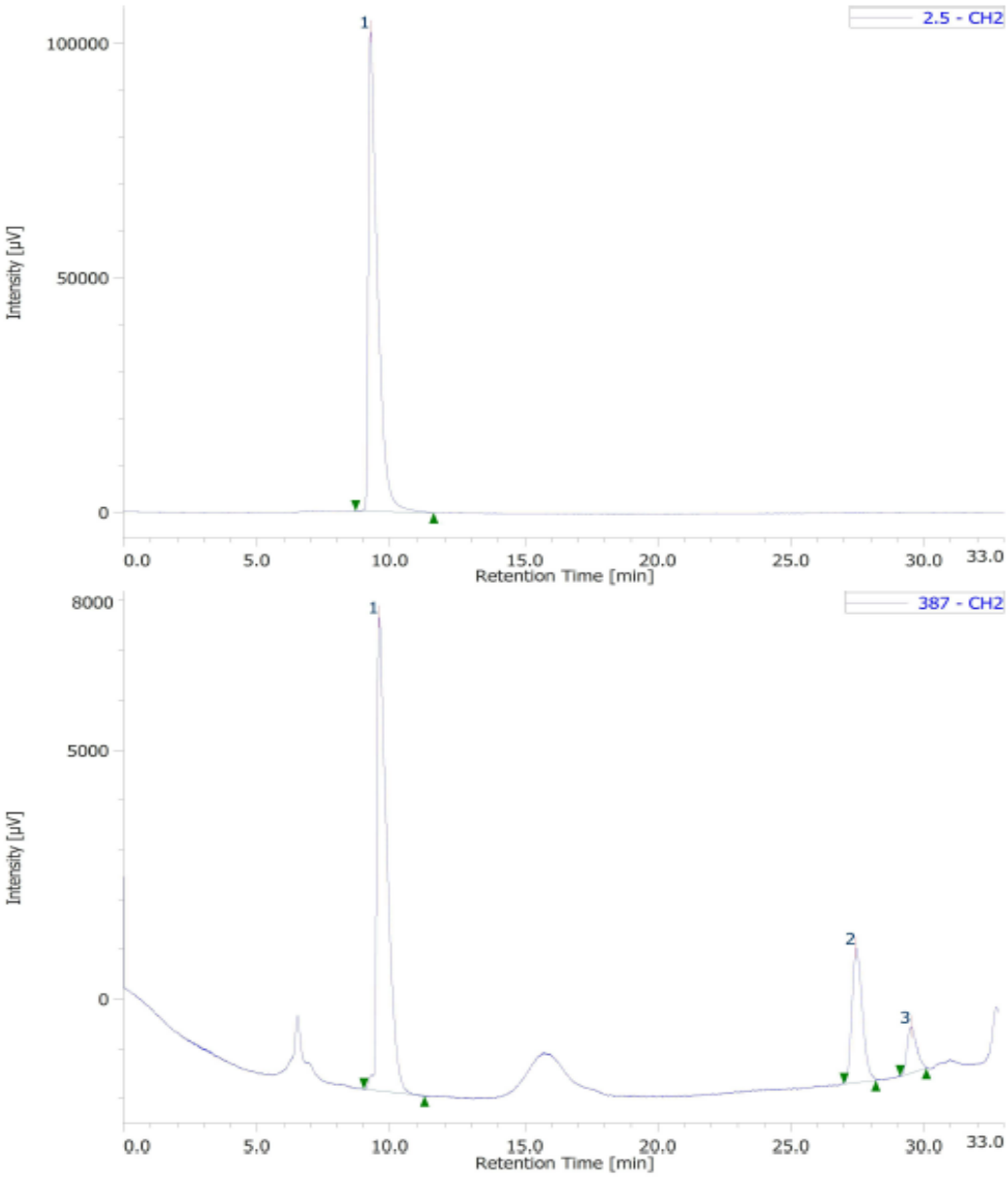

Figure 3: The upper graph indicates HPLC chromatogram of a standard of 5-methyl-tetrahydrofolic acid (5-CH3-H4folate). In the lower graph, an example of HPLC chromatogram of a strawberry sample.

4.6 Statistical analyses

Statistical analyses were performed using the software "Statistica 7" (Stasoft, Tibco Software, Palo Alto, California, USA). The data were analysed using one way

analysis of variance (ANOVA), while means were compared using the Student-Neumann-Keuls (SNK) test, with $p<0.05$. Results are expressed as mean ± standard error (SE).

5. RESULTS AND DISCUSSIONS

5.1. Effect of EU cultivation areas on fruit folate content of commercial cultivars

The results of the effect of different environments on the quality traits of well-established strawberry, raspberry and black currant cultivars are indicated as below. Starting with the strawberry samples, the range of folate concentration measured in this study for the different fruit samples of strawberry cultivars vary in the range of 30.37 to 84.58 ug/100g FW, for all CIREF samples. Similarly, the lone NIBIO variety (6_B), has fruit folate concentration of 43.16 ug/100g FW as seen in **figure 4**. The highest folate concentration is seen in fruits of cultivar 1_A (84.58 ug/100g FW), followed by cultivars 1_B and 5_B, with respective concentrations of 72.83 ug/100g FW and 73.43 ug/100g FW. The lowest folate levels are in fruits of cultivar 2_A. Thus cultivar 1_A with the highest value is well adapted to the French environment compared to other cultivars of strawberries.

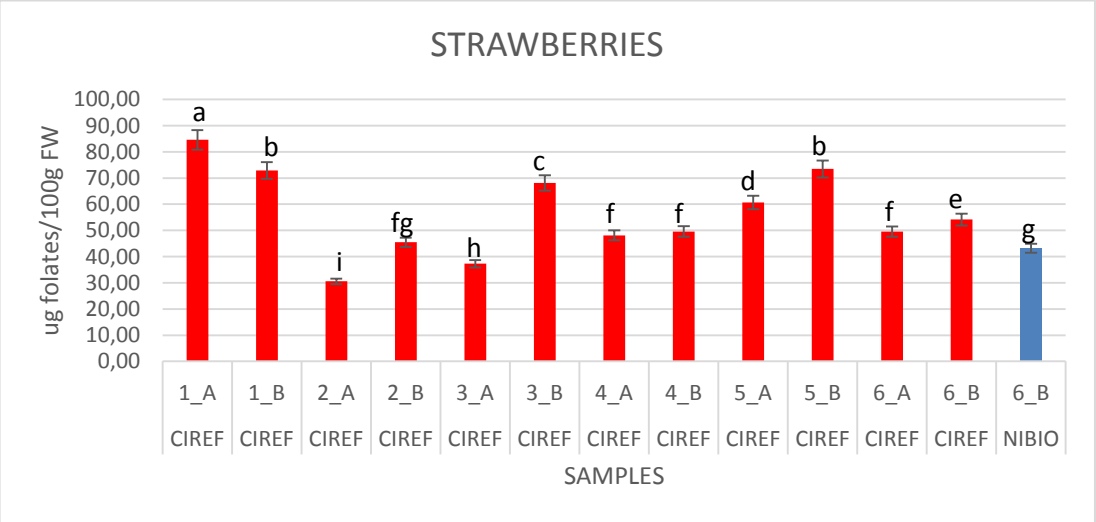

Figure 4: Folate content in 13 different samples of strawberry cultivars analysed in this study, expressed in ug/100g FW. Values are expressed as means ± standard errors. Histograms with different letters are statistically different (SNK Test, $p<0.05$).

Regarding the raspberry cultivar, the values of fruit folate concentration registered in this study ranges from 52.67 to 65.84 ug/100g FW, for CIREF samples. The folate values for HGU samples ranges from 17.01 to 24.59 ug/100g FW. Similarly, the folate values for NIBIO samples ranges from 18.80 to 21.26 ug/100g FW except

fruits of cultivar 2_A which showed a significant higher value of folate than the other cultivar from the same site. Cultivar 4_B from CIREF has the highest folate concentration of 65.84 ug/100g FW, followed by cultivar 2_B from NIBIO (61.53 ug/100g FW). Contrary, cultivar 1_A and 2_A from NIBIO have similarly low values with variety 1_B, from HGU being the lowest as seen in **figure 5**. In this light, variety 4_B from CIREF and 2_B from NIBIO showed a better environmental adaptability and quality traits as far as folate is concern in their respective environments.

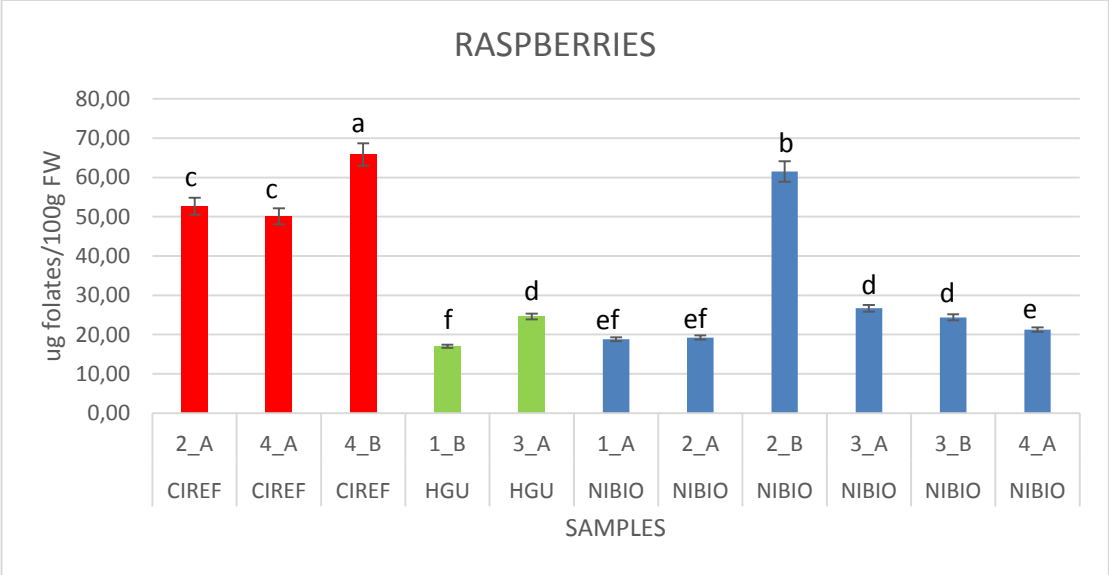

Figure 5: Folate content in 11 different samples of raspberry cultivars analysed in this study, expressed in ug/100g FW. Values are expressed as means ± standard errors. Histograms with different letters are statistically different (SNK Test, $p<0.05$).

In the case of black currant, the fruit folate concentration registered in this study, all obtained from, ranges from 12.01 to 24.69 ug/100g FW, as seen on **figure 6**. The cultivar BC_11, BC_10 AND BC_1 showed the highest values of folate concentration, with BC_11 having the highest value. Contrarily, cultivars or samples BC_8, BC_5 and BC_2 showed lower values with BC_8 being the lowest and indicating poor adaptability to this environment, in terms of fruit folate content.

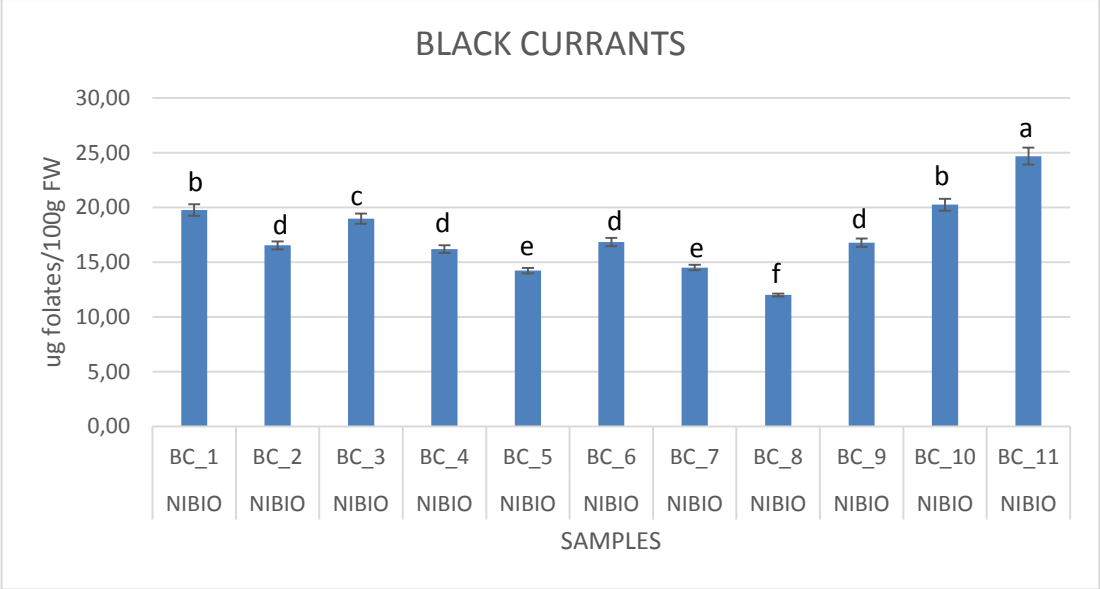

Figure 6: Folate content in 11 different samples of Black currant cultivars analysed in this study, expressed in ug/100g FW. Values are expressed as means ± standard errors. Histograms with different letters are statistically different (SNK Test, $p<0.05$).

5.2 Effect of EU cultivation areas on fruit folates content of strawberry breeding selections

This work focused on the evaluation of quality traits 126 strawberry selection (F1 generation), originating from Sennga Senngana and Candonga, which are adapted to different areas (Northern and Southern Europe). Each selection or genotype showed a unique quality trait in terms of folate content as follows;

Starting with CIREF selections, the levels of fruit folate concentration registered in the different strawberry selections, ranges from 12.82 to 78.19 ug/100g FW (**figure** 7). The selections H070, H047 and H018, showed the higher levels of folate content, with selection H018 being the highest, but slightly below cultivar 1_A of the same CIREF environment previously showed. Contrarily, selections H087, H012, H008 showed lower levels of folate concentration, with H087 being the lowest in terms of folate content, also in comparison to cultivar 2_A. This implies that established strawberry cultivars are well adapted to the CIREF environment in terms of folate content compared to strawberry selections originating from Senga Sengana and Candonga.

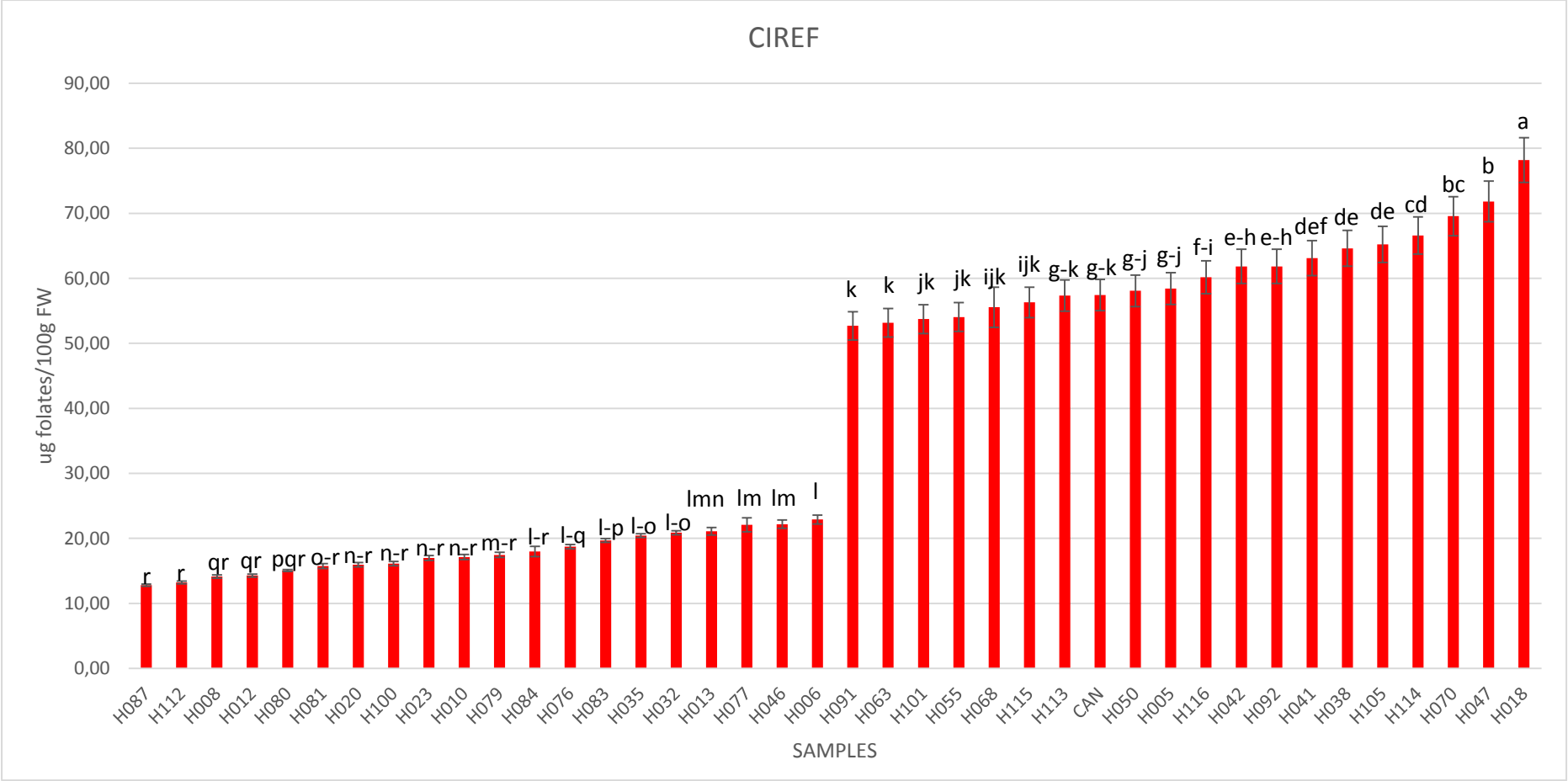

Figure 7: Folate content in fruits of the best 20 and the worst 20 selections of strawberries cultivated at CIREF, expressed in ug/100g FW. Values are expressed as means ± standard errors. Histograms with different letters are statistically different (SNK Test, $p<0.05$).

In the case of HANSABRED, the folate content varies from 14.27 to 123.40 ug/100g FW as seen in **figure 8**. Selections H050, H031, H027 showed higher levels of fruit folate content, with H027 being the highest followed by H050 and H031 respectively. Contrarily, selections H010, H040, H088 recorded the lowest levels of folate contents with no significant difference.

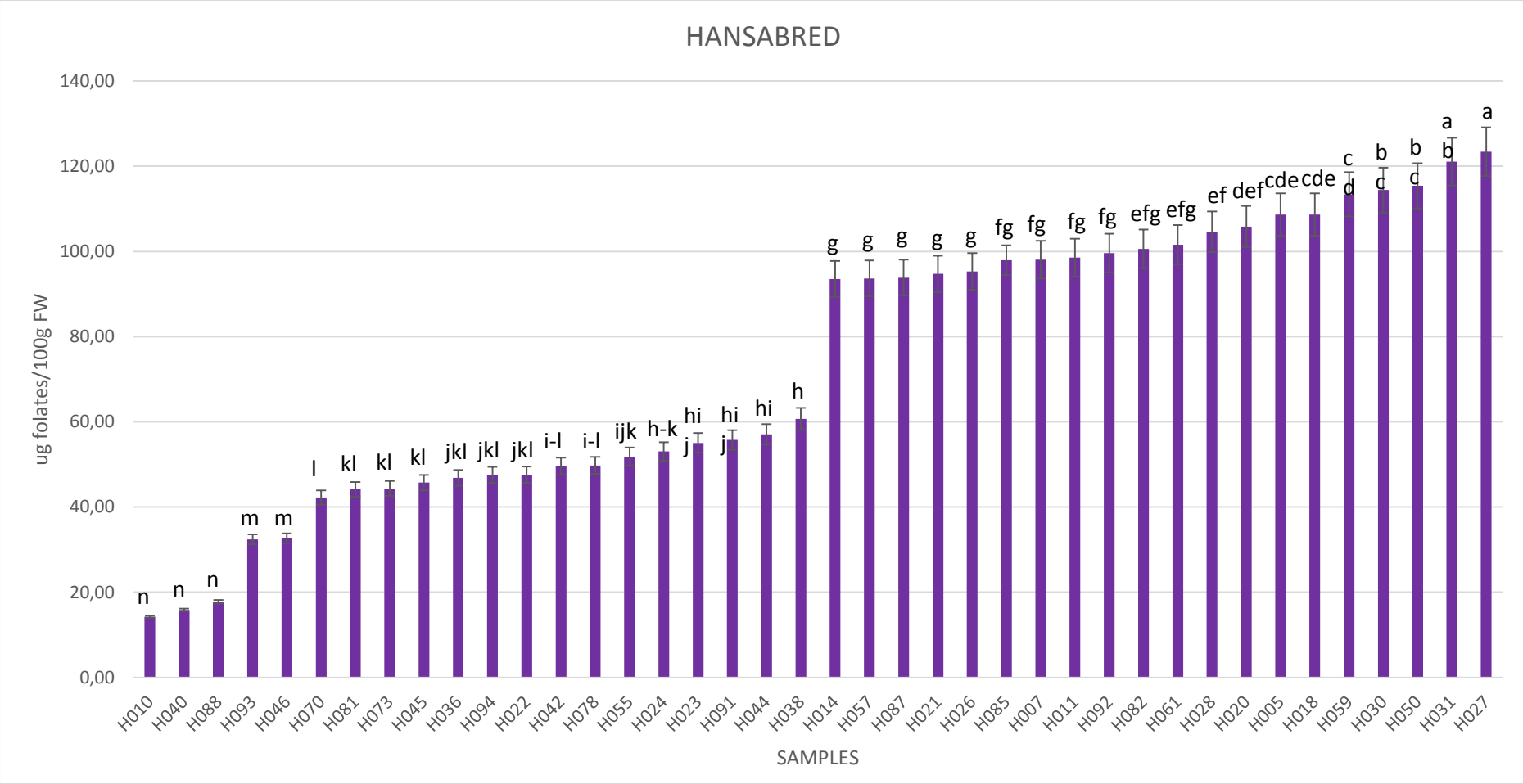

Figure 8: Folate content in fruits of the best 20 and the worst 20 selections of strawberries cultivated at HANSABRED. expressed in ug/100g FW. Values are expressed as means ± standard errors. Histograms with different letters are statistically different (SNK Test, $p<0.05$).

Regarding IFAPA, the folate levels in the different selections of strawberry fruits vary in the range 28.03 to 92.79 ug/100g FW. The higher levels of folate content were registered in selections H122, H048 and H085, with H085 being the highest, followed by H048 and H122 as seen in **figure 9**. Contrarily, selections H036, H093 and H013 showed lower levels of folate content, with H036 being the lowest.

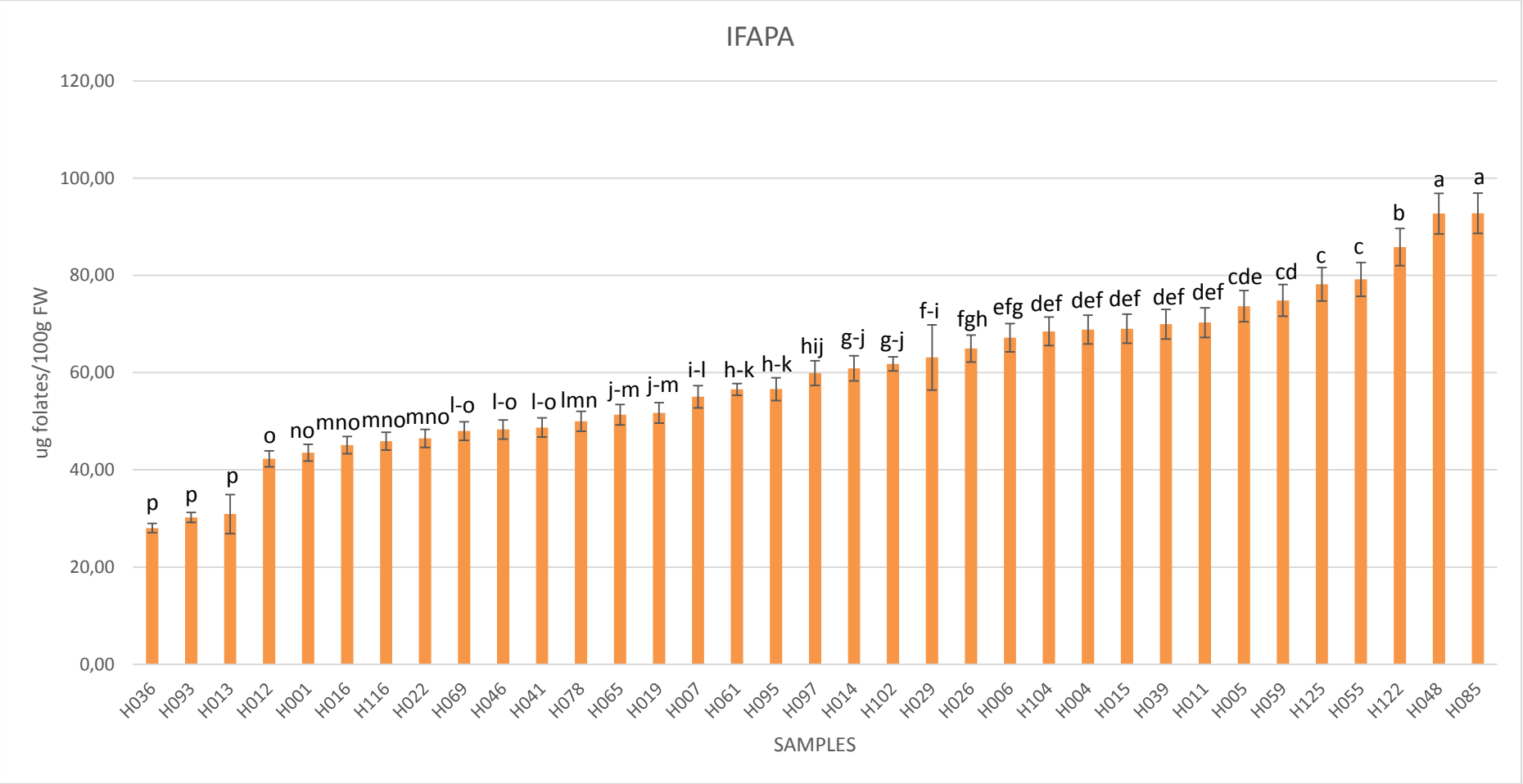

Figure 9: Folate content in fruits of 35 different selections of strawberries cultivated at IFAPA, expressed in ug/100g FW. Values are expressed as means ± standard errors. Histograms with different letters are statistically different (SNK Test, $p<0.05$).

Similarly, the UPM selections indicated folate content in range of 16.74 to 120.53 ug/100g FW. Selections H028, H018 and H120 showed higher fruit folate levels, with H120 being the highest, followed by H018 and H028 respectively (**figure 10**). Selection H018 and H120 show no significant difference in terms of folate content. Contrarily, the lower levels of folate content were registered in fruits of selections H035, H083 and H055, with H035 being the lowest.

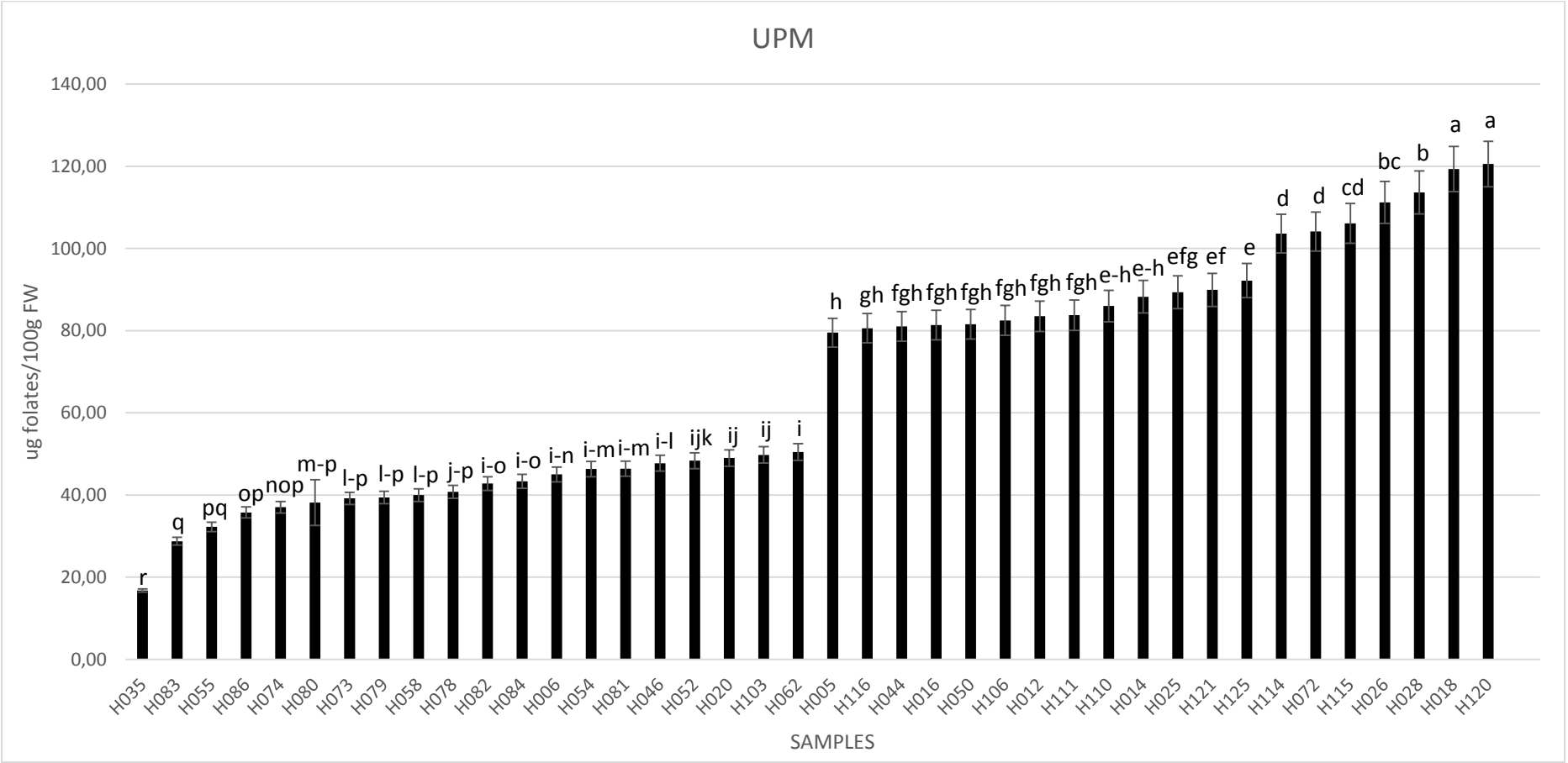

Figure 10: Folate content in fruits of the best 20 and the worst 20 selections of strawberries cultivated at UPM, expressed in ug/100g FW. Values are expressed as means ± standard errors. Histograms with different letters are statistically different (SNK Test, $p<0.05$).

Finally, the average levels of fruit folate content for the different selections in each environment was also demonstrated. It ranges from 38.30 to 66.22 ug/100g FW (**figure 11**). The highest level of folate content can be clearly seen in the selections originating from the HANSABRED. This also indicated that the environment of HANSABRED is the most favourable for the synthesis of folates in strawberry population, followed by UPM and IFAPA. Also, the highest folate levels among all the studied selections is from HANSABRED, and this confirms the influence of its pedoclimatic conditions on folate production. CIREF selections showed the lowest average folate levels, indicating the low nutritional quality, in terms of folate amounts, of the fruits from the selection cultivated in this site.

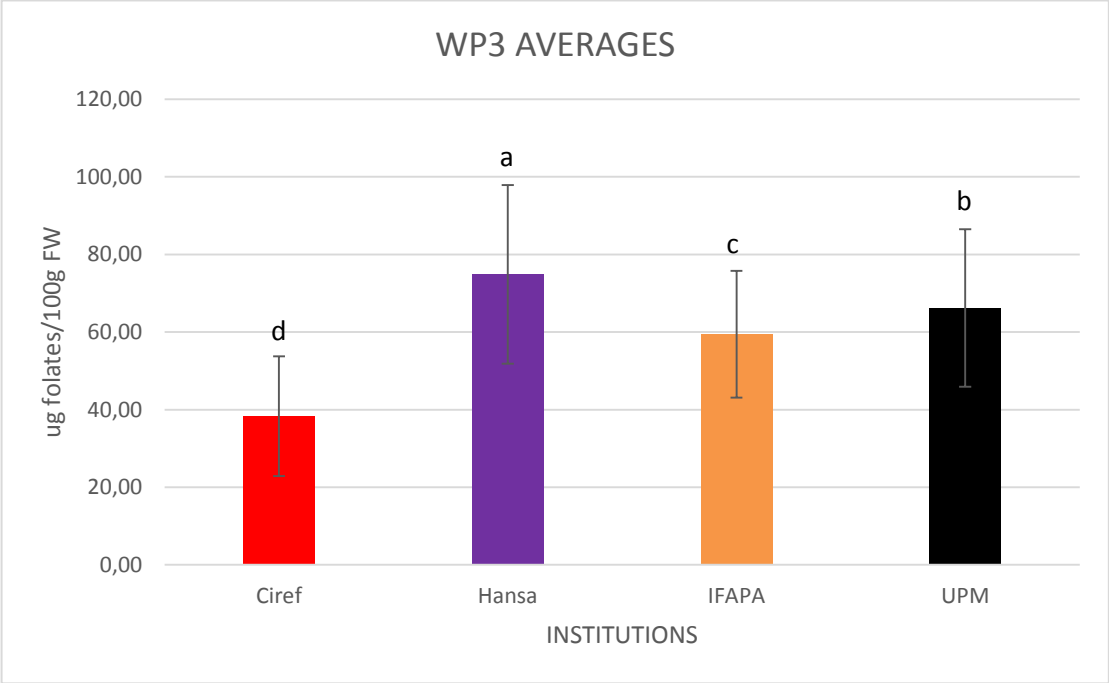

Figure 11: Fruit folate content on average bases of the different strawberry breeding selections in each of the research institution involved in this study, expressed in ug/100g FW. Values are expressed as means ± standard errors. Histograms with different letters are statistically different (SNK Test, $p<0.05$).

The influence of different cultivation locations on the fruit folate concentration can be demonstrated on **table 5** below. The folate values shown by the best 3 breeding selections chosen from each cultivation site confirms the fact that fruit folate content is highly influenced by cultivation condition/environment. In fact, among the different locations, there is not any common selections among the best three for

folates content, but each location has its own best selections for the folates amount. Only one selection, H018, performed well in two environments (UPM and CIREF).

Table 5: Fruits folate content of the 3 best breeding selections from each location

Selection	Environment	**Folate content in ug/100g FW**
H027	HANSABRED	123.40
H031	HANSABRED	121.08
H050	HANSABRED	155.42
H120	UPM	120.53
H018	UPM	119.32
H028	UPM	113.63
H085	IFAPA	92.78
H048	IFAPA	92.71
H122	IFAPA	85.86
H018	CIREF	78.19
H047	CIREF	71.82
H070	CIREF	69.56

CONCLUSION

The folate or Vitamin B9 content of strawberry, raspberry and black currant cultivars (WP1), and of 126 strawberry selections (F1 generation) resulting from a cross between two varieties, Senga Sengana and Candonga (WP3), cultivated in different European environments were evaluated using HPLC.

Among the 3 different species (strawberry, raspberry and blackcurrant), strawberry cultivar 1_A cultivated at CIREF showed the most interesting results, with the highest folate levels of 84.58 ug/100g FW. This cultivar could be the most adapted in his environment and could also serve as a parent for further breeding program of CIREF institution in order to enhance the folate quality of strawberries.

Cultivar 4_B (raspberry) still belonging to CIREF also showed interesting fruit folate levels of 65.84 ug/100g FW

The black currant cultivars showed the lowest results (24.69 ug/100g FW).

Regarding the 126 F1 strawberry breeding selections, its worth noting that the F1 population generated by crossing Senga Sengana and Candonga cultivars has demonstrated a large variation in fruit levels of folate conent. Fruits of H027 selection tested by HANSABRED in Germany showed the highest value of folate content (123.40 ug/100g FW). This is an indication that the HANSABRED environment is most suitable for the cultivation of F1 generation of strawberries in order to guarantee maximum strawberry quality with respect to folate or vitamin B9. This was followed by selections H120 from UPM and H085 from IFAPA with folates levels of 120.53 and 92.79 ug/100g FW respectively. Therefore, the HANSABRED and the UPM pedoclimatic conditions offers the best adaptability for strawberry fruit quality in terms of folates and the selections H027 (HANSABRED) and H120 (UPM) could possibly serve as parents for any future breeding program aimed at enhancing strawberry quality with respect to folate or vitamin B9.

Finally, this study demonstrated the influence of pedoclimatic conditions on the fruit folate concentration, as there is not any common selections among the best three for folates content for the different locations. Only one selection, H018, performed well in two environments (UPM and CIREF), and could be particularly interesting as folates source for further breeding programs in those environments.

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