Research Article Open Access

Drought stress affects nutritional and bioactive compounds in potatoes (*Solanum tuberosum* L.) relevant to human health

Christina B. Wegener, Hans-Ulrich Jürgens, Gisela Jansen

Julius Kuehn Institute (JKI); Institute for Resistance Research and Stress Tolerance, Rudolf-Schick-Platz 3, 18190 Sanitz, Germany

Corresponding Author: Christina B. Wegener, Dr, Julius Kuehn Institute; Institute for Resistance Research and Stress Tolerance, Rudolf-Schick-Platz 3, 18190 Sanitz, Germany

Submission Date: Aug 9, 2016; Acceptance date: January 27, 2017; Publication date: January 31, 2017

Citation: Wegener C.B., Jürgens H.U., Jansen G. Drought stress affects nutritional and bioactive compounds in potatoes (Solanum tuberosum L.) relevant to human health. *Functional Foods in Health and Disease* 2017; 7(1): 17-35

ABSTRACT

Background: Potatoes react very sensitively to drought during growth. Thus, appropriate plant stress responses may affect metabolites associated with the health quality of tubers.

Objective: The aim of this study was to determine the effects of drought stress (DS) on soluble sugars, starch, crude protein, minerals, free amino acids (AAs), and fatty acids (FAs).

Design: The experiment was carried out on three potato genotypes during two years with four replications. The plants were grown in pots in a glasshouse with optimal water supply and under drought stress conditions. After harvest, the tubers of these two variants were analyzed for nutritional and bioactive compounds relevant to human health.

Results: Apart from genotypic differences in most parameters, the results revealed that the DS caused a decline in glucose and fructose ($P < 0.05$, all) in both years, while sucrose was increased, especially in the second year with severe stress ($P < 0.01$). Starch was significantly reduced by moderate stress in the first year ($P < 0.01$), but less affected in the second year. Crude proteins and total amounts of free amino acids (AAs) were clearly enhanced by the stress in both years ($P < 0.05$, all). The minerals magnesium (Mg), potassium (K) and phosphorus (P) ($P < 0.05$, all) were similarly enhanced, while calcium (Ca) actually declined ($P < 0.05$). The portion of α-linolenic acid (ALA) on total lipids was elevated in the stress variants ($P < 0.01$), while oleic acid (OLA), its precursor, decreased significantly ($P < 0.05$), but only in the first year. In the second year, ALA was generally higher and not further induced by the stress. Additionally, OLA was less affected in that year, which was similar to all the other FAs in both years. Interestingly, *Myo*-inositol (MI)

and lipid acyl hydrolases (LAH) associated with modulation in cell membrane lipids were raised by the drought stress in each year ($P < 0.01$, all). In the second year, MI and LAH data of the drought stressed tubers correlated significantly ($r = 0.90$, $P < 0.01$), suggesting their joined action within plant stress responses.

Conclusions: The biochemical changes induced by DS are not alarming with regards to human health. Decline in glucose, fructose, and starch, in addition to increase in crude proteins, free AAs, ALA, MI, and minerals like Mg, K and P is profitable for the health benefits of tubers. However, a better quality is associated with a decrease in tuber yield.

Keywords: potato, drought stress, bioactive compounds, chronic disease

INTRODUCTION

Plant growth in nature is influenced by various biotic and abiotic stress factors. Drought is one of the most important stress factors affecting agricultural productivity around the world [1, 2, 3]. In the future, water will be the most limiting resource in crop production [4]. The combination of global warming with water scarcity will increase the frequency and severity of drought and endanger the natural resources [5]. In light of this context, it is important to study the impact of drought on plant growth and to also develop drought tolerant crops [4].

Plants have evolved specific mechanisms to cope with adverse environments leading to acclimations and adaptations. Better understanding of these mechanisms may increase the rate at which crop species can be improved [4] in the interest of productivity and quality. Drought stress (DS) induces a wide range of physiological and biological responses in plants. For instance, the accumulation of various metabolites like osmolytes and a set of protective proteins is involved in stress tolerance [6]. However, in first line drought triggers the phytohormone abscisic acid (ABA) which in turn initiates stomata closure and the expression of various stress related genes [6]. Moreover, the plant hormone jasmonic acid (JA) that regulates plant stress responses is up-regulated by exposure to DS [7]. Furthermore, the potato (*Solanum tuberosum* L.), which is one of the most important staple crops, is highly sensitive to DS. However, appropriate adaptive responses alter the biochemistry of the tubers. The consumption of these tubers can thereby be associated with positive as well as negative consequences for human health.

Potatoes contain significant amounts of protein (2% of fresh weight, FW), dietary fiber (1-2% of FW), amino acids, the vitamins C, B6, B1, the minerals potassium, phosphorus, magnesium and the micronutrients iron (0.43 mg/100 g FW), and zinc (0.34 mg/100 g FW) [8]. Potato protein has an excellent nutritional quality, as indicated by its high biological value (BV=79.5) [9] and essential amino acid index ($EAAI_{adult} = 101.4\%$) [10]. The tuber protein provides a good source of the essential amino acids lysine, leucine, phenylalanine, threonine, isoleucine, valine, and more [11],with its quality level being about 70% of a whole egg protein [10]. Furthermore, the tubers accumulate secondary metabolites, such as plant phenols [12] and anthocyanins [13], that all generate antioxidant activities [14], in addition to phytosterols (5.1 mg/100 g FW) [15] that can reduce intestinal cholesterol absorption and serum LDL-cholesterol levels [16].

Furthermore, potato is rich in starch (15-20% of FW) [17], being the main contributor to the dietary glycemic index (GI). Among 13 potato genotypes (GTs) listed in a database of the

University of Sydney, the GI-values for boiled tuber samples of 150 g ranged from 56 to 94 [18]. During digestion, starch breaks down quickly into glucose which is rapidly absorbed in the blood stream. Hence, carbohydrate-rich foods like potatoes play a role in postprandial glucose and insulin responses, and may be linked to chronic diseases such as diabetes and cardiovascular diseases (CVDs) [19] when too much is consumed. Associations between consumption of potatoes and diabetes mellitus have been reported [20, 21]. However, potato is also a good source of dietary resistant starch (RS), *i.e.* a form of starch that resists digestion in the small intestine. RS is seen as a type of dietary fiber conferring considerable benefits to human health, *e.g.* reduced glycemic response as well as improved colonic health [22, 23]. Chilled potatoes seem to have more RS than either hot or reheated tubers [24]. In first line, however, potato is an important energy source in the human diet. Due to their high starch contents, the tubers contribute to combat malnutrition, a primary cause of immunodeficiency. Nutritional deficiencies are associated with a poor immune response to diseases and play a role in morbidity and mortality worldwide [25]. Drought may trigger this malnutrition-infection cycle, especially in developing countries [26].

Therefore, it is important to obtain a better knowledge on drought-induced changes and their impact on the quality of tubers including the nutritional and bioactive compounds relevant to human health. A previous experiment was focused on plant phenols and antioxidants [27]. This study concentrated on soluble sugars and starch, in addition to *myo*-inositol (MI), crude protein, fatty acid (FA), several minerals, and free amino acids (AAs).

METHODS

Plant Material

Two purple breeding clones (St 89403 & St 3792) and one yellow fleshed cultivar Agave (cv.) were used in this study. The experiments were carried out with four replications in a randomized design during two years (2014/15). The plants were grown in pots (95% turf-sand mixture) from April to September in a greenhouse. Control plants were grown with sufficient water supply (1. variant) and the application of drought stress started seven weeks after planting (2. variant), *i.e.* with the initiation of tuber bulking. At that time, the water supply was fully stopped for six days. Afterwards, each plant received 50 ml of water per day, an amount that was further reduced up to 30 ml from the middle of August to the end of cultivation. In the second year, supply with only 30 ml of water started already in the middle of July and was reduced up to 20 ml from the middle of August to the end. These two watering regimes enabled different drought intensities, *i.e.* moderate stress in the first and severe stress in the second year. The mean temperature (°C) during the growing season of the first and second year was as follows: May, 9.8/7.9; June, 12.5/10.8; July, 19.8/17.1; August 16.1/18.9, September, 15.3/13.2. In both years, the standard recommended rates for commercial fertilizer (NPK + trace elements) and pesticides were applied. After harvest, the yield was determined and the tubers of both variants were stored under controlled environment until the tissue samples were taken for the analyses as detailed below.

Ten medium sized tubers were randomly taken from each genotype replicated as an average sample. The tubers were washed, air-dried, and then cut into halves using a knife. Next, a 5 mm thick tissue section was excised from each half before 50 g of tissue slices were weighed, dried in a freeze dryer Alpha 1-4 LD plus (Christ, Osterode Germany), and then ground in a laboratory mill SM3 equipped with a fine sieve (Brabender, Duisburg, Germany). The freeze-dried tissue

powder was used for the analyses which began in November and finished in December of each test year.

Analyses

The soluble sugars glucose, fructose, and sucrose as well as *myo*-inositol were analyzed by gas chromatography according to Niederer *et al.* [28] on an Agilent 5890A gas chromatograph (GC) with FID (Agilent Technologies, USA). The *Ewers* method [29] was used for the analyses of starch on an automatic polarimeter, Autopol I from Rudolf Research Analytical, USA. The analytical block digestion system Kjeldatherm®, the distillation system VAPODEST® both from C. Gerhard, Germany and the TitroLine easy apparatus from Schott Instruments, Germany were combined for the determination of crude protein by means of the *Kjeldahl* method [30]. Lipid acyl hydrolase (LAH) enzyme activity was measured on a UV spectrophotometer from Kontron Instruments, Germany according to Bohac [31] with modifications [32]. Extraction of total tuber lipids was performed as detailed by Bligh and Dyer [33] before fatty acids were analyzed as fatty acid methyl ester (FAME) on an Agilent 6890A GC with FID (Agilent Technologies, USA) as described by Arens *et al.* [34]. Inductively coupled plasma optical emission spectroscopy (ICP-OES) was used for the assay of calcium (Ca), phosphorus (P), magnesium (Mg) and potassium (K) on an ICP spectrometer iCAP 6000 (ThermoScietific, USA) following methods which are described in details elsewhere [35]. Total amounts of free amino acids were analyzed by high-performance liquid chromatography (HPLC) according to Cohen & Michaud [36], and adapted to a LUNA C 18 (2) bonded silica column (Phenomenex, Germany), as described by Hernández-Orte *et al.* [37]. All analyses were carried out in triplicate or at least in duplicate. The data presented in the tables 2ABC, 3A, 4AB and 5AB are expressed on a dry matter (DM) basis.

Standard statistical methods were used for data analyses. Mean values ± SD (Standard deviation) are presented in the tables. Data were subjected to two-way analysis of variance (ANOVA) and PROC GML procedure SAS, Tukey test at the 0.05 level (SAS 9.2 statistical package, SAS Institute, USA) was used to assess effects of drought stress. Pearson correlation was applied to study associations between the parameters. $P \leq 0.05$ was regarded to be statistically significant.

RESULTS AND DISCUSSION

Tuber yield

The first test year was characterized by moderate drought stress (1) and the second year by severe stress (2). Both types of DS caused a significant decrease of tuber yield (Table 1). The yield loss was significantly higher under severe DS in the second year, compared to moderate stress in the first year. St 3792 had the lowest yield reduction in every year, while St 89403 was most sensitive to DS and revealed the strongest yield loss (Table 1).

The decline in tuber yield of all genotypes confirms that the drought stress was successfully applied in every year, thereby enabling the study of its impact on the nutritional and bioactive compounds in tuber tissue. This notion is underlined by a strong boost in proline as a stress related marker discussed below

Table 1 Tuber yield of the control and drought stress variants and percentage of reduction

Genotype	Tuber yield (g per plant)		
	Control	Stress	Reduction (%)
Year (1)			
St 89403	134.83 ± 5.90ab	89.22 ± 7.27a	33.8**
St 3792	127.13 ± 6.60a	103.20 ± 4.64ab	18.8**
Agave	148.97 ± 9.17b	113.96 ± 3.92b	23.5**
Average	136.98 ± 11.66	102.13 ± 11.51	25.4***
Year (2)			
St 89403	156.93 ± 8,53a^{I}	82.99 ± 9,17a	47.1**
St 3792	166.76 ± 11,78a^{I}	123.55 ± 2.16b^{II}	25.9**
Agave	201,21 ± 7.90b^{II}	132.28 ± 4.60c^{II}	34.3**
Average	174.97 ± 21.26^{III}	112.94 ± 22.31^{I}	35.7***

a,b,c Genotype means with different letters within a column are significantly different at $P \leq 0.05$. Differences between the control and drought stress variants are significant at $^{**}P \leq 0.01$ and $^{***}P \leq 0.0001$. Differences between the years are significant at $^{I}P \leq 0.05$, $^{II}P \leq 0.01$ and $^{III}P \leq 0.0001$.

Carbohydrates

Carbohydrates (CHO) are major energy sources and contribute to the dietary glycemic index [38]. Additionally, potatoes contain significant amounts of CHO, mainly starch [8, 17]. The results reveal that DS has a clear effect on the CHO tested in this work (Tables 2A-2C).

Glucose and fructose were significantly reduced by drought stress (Table 2A). This was discovered in both years when all genotypes were regarded. On average, the decrease in glucose ranged from 54.2% in the first year (1) to 69.3% in the second year (2), and that in fructose ranged from 54.3% (1) to 56.2% (2).

Sucrose was enhanced on average by the drought stress (Table 2B), while its raise was significantly stronger in the second year (+111.1%) compared to the first year (+7.9%). In particular, purple clones had multiple higher sucrose levels in their drought stressed tubers (St 89403, 3.4-fold; St 3792, 1.6-fold), while cv. Agave was less affected by severe drought in the second year. Under moderate stress in the first year, increase in sucrose was not statistically significant.

Total soluble sugar (TSS) levels decreased significantly (-25.2%) as a result of moderate stress applied in the first year (Table 2B), a tendency found within all GTs. In the second year, similarly reduced TSS could only be detected for cv. Agave, while purple clones exhibited significantly higher TSS values as a result of severe stress. Although in that year, TSS were in general on a lower level in tubers of purple clones. Therefore, they were probably induced by the stress (Table 2B). This possibly suggests that potato genotypes react differently and highly sensitive in their TSS contents on the DS. Nevertheless, it may be possible to find GTs with smaller amounts of sugars under such conditions, *e.g.* cv. Agave.

Table 2A. Contents of glucose and fructose in tubers grown under control and drought stress conditions

Genotype	Glucose (mg g^{-1})		Fructose (mg g^{-1})	
	Control	Stress	Control	Stress
Year (1)				
St 89403	5.82 ± 1.43a	1.56 ± 0.58a**	5.78 ± 1.69a	1.89 ± 0.83a**
St 3792	2.89 ± 0.60b	1.98 ± 0.43a	4.18 ± 0.68a	2.58 ± 0.49a*
Agave	5.83 ± 1.96a	3.11 ± 1.11a	4.61 ± 1.06a	2.20 ± 0.52a*
Average	4.85 ± 2.00	2.22 ± 1.01**	4.86 ± 1.40	2.22 ± 0.69**
Year (2)				
St 89403	3.14 ± 0.60a	0.60 ± 0.08a^{*Ⅰ}	2.58 ± 1.29a	0.51 ± 0.09a^{*Ⅰ}
St 3792	2.13 ± 0.38a	2.19 ± 0.58b	1.15 ± 0.27a^{Ⅰ}	1.48 ± 0.49b
Agave	11.63 ± 4.88b	2.41 ± 0.57b*	2.57 ± 1.63a	0.76 ± 0.43a^{Ⅰ}
Average	5.63 ± 5.11	1.73 ± 0.94*	2.10 ± 1.38^{Ⅱ}	0.92 ± 0.56^{*Ⅱ}

a,b Genotype means followed by different letters in the same column differ significantly at $P \leq 0.05$. Difference between control and drought stressed tubers is significant at $^{*}P \leq 0.05$ and $^{**}P \leq 0.01$. Differences between the years are significant at $^{Ⅰ}P \leq 0.05$ and $^{Ⅱ}P \leq 0.01$.

Starch contents were significantly decreased by moderate drought in the first year, *i.e.* -4.3% on average (Table 2C). In the second year, both purple clones had less starch in their control tubers than in the first year ($P < 0.05$); Similarly, as cv. Agave they were not significantly affected in this respect by severe DS (Table 2C).

With regards to health quality, these are positive findings because starch as major CHO in tubers contributes to the glycemic index of potato [38, 39]. Diets with a high glycemic load are linked to higher risk of non-communicable diseases (NCD) like type 2 diabetes and CVDs, whereby postprandial hyperglycemia plays a significant role in the disease progress [19]. In this frame, reduction in glucose and fructose by drought stress (Table 2A) is another good result. CHO, especially soluble sugars are rich in energy and support weight gain. Excessive consumption of CHO, *e.g.* with sweets, cakes, and beverages is a main factor, besides little to no sportive activity, in the development of human obesity, a condition which has increased dramatically worldwide [38]. Adiposity enhances the risk of diabetes, hypertension, CVDs, inflammatory disorders, renal failure, and cancer [19, 38]. Nevertheless, reduced CHO contents are less optimal in the case of malnutrition and diseases associated with it [25, 26].

Table 2B. Contents of sucrose and total soluble sugars in tubers grown under control and drought stress conditions

Genotype	Sucrose (mg g^{-1})		Total soluble sugars (mg g^{-1})	
	Control	Stress	Control	Stress
Year (1)				
St 89403	10.30 ± 1.23a	9.92 ± 0.44a	21.91 ± 3.25a	13.37 ±1.51ab**
St 3792	10.76 ± 0.74a	12.48 ± 2.02a	17.90 ± 1.49ab	17.05 ± 2.80a
Agave	4.69 ± 0.31b	5.37 ± 0.31b	15.13 ± 3.02b	10.69 ± 1.50b
Average	8.58 ± 2.89	9.26 ± 3.18	18.31 ± 3.88	13.70 ± 3.30**
Year (2)				
St 89403	6.40 ± 3.06ab	21.85 ±1.84a^{*H}	12.13 ± 5.68a^{I}	22.95 ± 2.01a^{*I}
St 3792	7.57 ± 2.30a	12.01 ± 1.48b*	10.86 ± 2.91a^{I}	15.68 ± 1.56b*
Agave	4.10 ± 0.18b^{I}	4.28 ± 0.32c^{I}	18.30 ± 7.46a	7.46 ± 0.66c^{*I}
Average	6.02 ± 2.64^{I}	12.71 ± 7.32**	13.76 ± 6.21^{I}	15.36 ± 6.51

a,b,c Genotype means followed by different letters in the same column differ significantly at $P \leq 0.05$. Difference between control and drought stressed tubers is significant at $^{*}P \leq 0.05$ and $^{**}P \leq 0.01$. Differences between the years are significant at $^{I}P \leq 0.05$ and $^{H}P \leq 0.01$.

Protein, Myo-Inositol and Fatty Acids

Crude protein was significantly enhanced due to drought stress, on average by 30.3% in the first year and 26.4% in the second year (Table 2C). St 3792 exhibiting the lowest yield loss among the GTs (Table 1) had the highest contents of crude protein in control and drought stressed tubers. Addtionally, this purple clone generated the strongest increase in protein with up to 35.0% under severe drought in the second year. This was not surprising as proteins are important constituents of cellular membranes and various cytoplamic structures [40], and protective proteins are often induced within plant stress responses [6].

Additionally, a boost of crude protein is positive for human health as potato protein has high nutritional value. It contains several essential amino acids (EAA), *e.g.* lysine, leucine, valine, isoleucine and phenylalanine, so that the essential amino acid index of tuber protein is relatively high for plant protein [10, 11]. Patatin, a family of glycoproteins with a molecular weight of about 40 - 43 kDa comprises 40% of the soluble protein in potatoes [31], and is a good source of amino acids and patatin also generates antioxidant activities [41].

Additionally, potato patatin exhibits lipid acyl hydrolase (LAH) activity [42]. LAHs are lipolytic enzymes involved in changes of membrane lipids and release of fatty acids [43], which are associated with plant stress responses [42]. The results of this study revealed significantly higher LAH activities in drought stressed tubers than in the control tubers (Table 3A). An increase in LAH by DS was consistently found with all GTs, in the first (+42.8%) and second year (+54.7%), which supports the results of previous experiments [32].

Myo-inositol, a sugar alcohol, was also elevated by the stress (Table 3A). Significantly higher MI contents due to DS were detected for all GTs in the first (+95.5%) and second year (+107.1%). MI is involved in cell wall biosynthesis, plant growth, and signaling, while also being a component

of membrane phospholipids. In cellular membranes, inositol phospholipids (IP) play a role in signaling pathways that modulate a wide range of cellular functions essential for adaptation to a changing environment [44, 45]. Consequently, the raise of *myo*-inositol due to drought stress was not surprising. Moreover, it is interesting that within the tubers grown under severe stress in the second year, the LAH and MI data were significantly correlated ($r = 0.90$, $P < 0.01$), a fact indicating a concerted action in plant adaptive responses.

Table 2C. Contents of starch and crude protein in tubers grown under control and drought stress conditions

Genotype	Starch (g 100 g^{-1})		Crude protein (g 100 g^{-1})	
	Control	Stress	Control	Stress
Year (1)				
St 89403	82.29 ± 2,71a	76.08 ± 0.90a**	4.72 ± 0.32a	6.26 ± 0.25a**
St 3792	75.00 ± 1.59b	71.49 ± 0.82b*	6.54 ± 0.92b	8.66 ± 0.71ab*
Agave	74.19 ± 0.55b	73.91 ± 0.44c	6.45 ± 0.38b	8.15 ± 0.43b*
Average	77.16 ± 4.08	73.83 ± 2.02**	5.90 ± 1.04	7.69 ± 1.15***
Year (2)				
St 89403	73.22 ± 0.73a^{I}	72.91 ± 1.32a^{I}	5.76 ± 0.64a^{I}	6.48 ± 0.31a
St 3792	70.42 ± 1.32a^{I}	73.22 ± 0.73a	7.39 ± 0.69b	9.98 ± 1.00b**
Agave	74.77 ± 2.25b	75.01 ± 0.40b^{I}	7.18 ± 0.62b	9.22 ± 0.42b**
Average	72.80 ± 2.39^{I}	73.71 ± 1.23	6.77 ± 0.97^{H}	8.56 ±1.64^{***H}

a,b,c Genotype means with different letters within a column are significantly different at $P \leq 0.05$. Difference between control and drought stressed tubers is significant at $^{*}P \leq 0.05$, $^{**}P \leq 0.01$ and $^{***}P \leq 0.0001$. Differences between the years are significant at $^{I}P \leq 0.05$ and $^{H}P \leq 0.01$.

Additionally, *myo*-inositol plays significant roles in human health. Thus, the raise of MI is desirable. For example, IP species act as membrane bound signaling molecules implicated in various processes of cellular physiology including metabolism, cellular growth, proliferation and survival. Moreover, disruption within this signaling pathway can be linked to diverse cancer, inflammatory disorders, obesity, and diabetes [46]. MI even has therapeutic effects, *e.g.* in treating diabetic neuropathy and as a lipotrophic factor it can reduce fat build up in the organs and with it the risk of intestinal lipodystrophy [47] or it serves as natural insulin sensitizer and mediates hormonal effects in women with polycystic ovary syndrome [48, 49, 50]. Moreover, inositol generates a calming and anti-depressant effect [51], and together with inositol hexaphosphate (IP_6) it might contribute to cancer inhibition [52].

Fatty acid composition of tuber lipids is another interesting aspect in this frame, although potatoes contain little fat with 0.1 - 0.2% of the FW. A major fraction of tuber lipids are membrane lipids, mainly phosphatidylcholine and phosphatidylinositol [53].

Table 3A. Lipid acyl hydrolase (LAH) activity and contents of *myo*-inositol in tubers grown under control and drought stress conditions

Genotype	LAH activity (U mg^{-1} min^{-1})		*Myo*-inositol (mg g^{-1})	
	Control	Stress	Control	Stress
Year (1)				
St 89403	3.46 ± 0.73a	5.21 ± 0.24a*	0.25 ± 0.01a	0.41 ± 0.02a**
St 3792	1.49 ± 0.13b	1.90 ± 0.13b**	0.27 ± 0.02a	0.33 ± 0.06a
Agave	1.72 ± 0.32b	2.41 ± 0.22b*	0.14 ± 0.02b	0.56 ± 0.08b**
Average	2.22 ± 1.00	3.17 ± 1.47**	0.22 ± 0.06	0.43 ± 0.11**
Year (2)				
St 89403	2.67 ± 0,13a	3.89 ± 0.56a^{*I}	0.21 ± 0.02a	0.72 ± 0.04a***
St 3792	1.42 ± 0.47b	2.15 ± 0.27b*	0.20 ± 0.02a	0.29 ± 0.01b**
Agave	2.61 ± 0.49a^{I}	4.31 ± 0.29a^{*I}	0.45 ± 0.03b	0.74 ± 0.12a*
Average	2.23 ± 0.70	3.45 ± 1.02***	0.28 ± 0.12	0.58 ± 0.22^{**I}

a,b Genotype means followed by different letters in the same column differ significantly at $P \leq 0.05$. Difference between control and drought stressed tubers is significant at $^{*}P \leq 0.05$, $^{**}P \leq 0.01$ and $^{***}P \leq 0.0001$. Differences between the years are significant at $^{I}P \leq 0.05$.

The results of this study revealed that linoleic acid (LA) followed by palmitic and α-linolenic acid (ALA) had the highest portion on total tuber lipids (Table 3B). In the first year, ALA was significantly increased due to drought on average (+5.8%) and in all genotypes, while oleic acid (OLA), its precursor, decreased (-21.7%). Moreover, in St 3792 in the first year (Table 3C). A similar clear change of the two FAs was not found in the second year. All other FAs were less affected by DS in both years (Table 3B). LA and ALA are polyunsaturated FAs (PUFAs) and serve as precursors for oxylipins like jasmonates, *e.g.* jasmonic acid (JA), a plant hormone with signaling functions within plant stress responses [54]. In fact, high contents of PUFAs in membranes increase the membrane fluidity mitigating effects of environmental stresses [55]. Thus, the elevated α-linolenic acid contents under conditions of moderate stress in the first year were not surprising. In the second year, the ALA levels were in general higher in the non-treated control tubers and probably therefore not further induced by the stress.

Alpha-linolenic acid (18:3n-3) supports the health quality of tubers. Together with linoleic acid, ALA is essential in the human diet and can be converted partially into the longer chain ω-3 FAs eicosapentaenoic (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 20:6n-3) that are both required for better tissue functions [56].

EPA and DHA are seen to be linked with anti-inflammatory processes, better cardiovascular health and reduced risk of Alzheimer disease [57]. Additionally, ω-3 FAs, above all ALA contribute to the structure and function of the brain (about 33% of FAs in the brain belong to the omega-3 family), and there are reports that sufficient long chain PUFAs, such as DHA can reduce the risk of depression [58]. Therefore, increase in ALA observed in the first year (Table 3C) can be seen as a positive change.

Table 3B. Fatty acid composition of tuber lipids under control and drought stress conditions averaged over three genotypes

Fatty acids (%)				
Fatty acid type	Year (1)		Year (2)	
	Control	Stress	Control	Stress
Saturated				
Arachidic acid, C20:0	1.30 ± 0.20	1.42 ± 0.17	1.18 ± 0.28	1.23 ± 0.20^{I}
Behenic acid, C22:0	0.95 ± 0.06	0.97 ± 0.09	0.58 ± 0.12^{III}	0.63 ± 0.12^{III}
Lignoceric acid, C24:0	1.01 ± 0.19	0.94 ± 0.28	0.45 ± 0.28^{III}	0.47 ± 0.09^{III}
Myristic acid, C14:0	0.48 ± 0.09	0.47 ± 0.07	0.40 ± 0.06^{II}	0.41 ± 0.05^{II}
Palmitic acid, C16:0	21.64 ± 1.37	21.25 ± 1.16	21.59 ± 1.09	21.32 ± 1.19
Stearic acid, C18:0	5.68 ± 0.82	5.67 ± 1.11	5.07 ± 0.82^{II}	5.14 ± 1.02^{III}
Monounsaturated				
Eicosenoic, C20:1	nd	nd	0.48 ± 0.06	0.44 ± 0.08
Oleic acid, C18:1	1.57 ± 0.49	$1.23 \pm 0.35^{*}$	1.24 ± 0.12	1.20 ± 0.20
Vaccenic acid, C18:1	0.83 ± 0.13	0.82 ± 0.11	0.80 ± 0.14	0.77 ± 0.13
Polyunsaturated				
Linoleic acid, C18:2	51.99 ± 1.78	51.74 ± 1.86	52.15 ± 1.64	52.70 ± 0.90
Linolenic acid, C18:3	14.57 ± 0.87	$15.42 \pm 0.97^{**}$	16.06 ± 1.25^{II}	15.68 ± 0.89

Difference between control and drought stressed tubers is significant at $^{*}P \leq 0.05$ and $^{**}P \leq 0.01$. Difference between the years is significant at $^{I}P \leq 0.05$, $^{II}P \leq 0.01$ and $^{III}P \leq 0.0001$. nd, not detected. Mean ± SD, averaged over 3 genotypes.

Table 3C. Portion of linolenic and oleic acid on total FAs under control and drought stress conditions

Genotype	Linolenic acid (%)		Oleic acid (%)	
	Control	Stress	Control	Stress
Year (1)				
St 89403	15.63 ± 0.05a	16.40 ± 0.82a	1.55 ± 0.38a	1.54 ± 0.21a
St 3792	14.41 ± 0.29b	$15.33 \pm 0.47ab^{*}$	1.77 ± 0.63a	1.08 ± 0.37ab
Agave	13.63 ± 0.37c	$14.51 \pm 0.32b^{*}$	1.39 ± 0.34a	1.07 ± 0.21b
Average	14.57 ± 0.87	$15.41 \pm 0.97^{**}$	1.57 ± 0.49	$1.23 \pm 0.36^{*}$
Year (2)				
St 89403	15.40 ± 1.35a	15.12 ± 0.34a	1.33 ± 0.10a	1.47 ± 0.07a
St 3792	17.04 ± 0.67a	16.20 ± 1.10a	1.18 ± 0.09a	1.05 ± 0.05b
Agave	15.73 ± 0.93a	15.73 ± 0.68a	1.22 ± 0.12a	1.08 ± 0.06b
Average	16.06 ± 1.25^{II}	15.68 ± 0.89	1.24 ± 0.12^{I}	1.20 ± 0.20

a,b,c Genotype means followed by different letters in the same column differ significantly at $P \leq 0.05$. Difference between control and drought stressed tubers is significant at $^{*}P \leq 0.05$ and $^{**}P \leq 0.01$. Difference between the years is significant at $^{I}P \leq 0.05$ and $^{II}P \leq 0.01$.

Minerals

Potato is an excellent source of minerals, especially potassium [53]. The results revealed that calcium, magnesium (Table 4A), phosphorus, and potassium (Table 4B) tested in this work were clearly affected by the stress. Ca was reduced on average by 11.4% (1. year) and 22.2% (2. year), while Mg (+15.6%; +11.5%), P (+9.0%; +5.5%) and K (+9.0%; +14.3%) contents raised significantly as a result of drought stress in both years (Tables 4A, 4B). It is important, that a drought-induced boost in potassium as the main mineral in tubers was found for all GTs in every year, except St 3792 in the second year (Table 4B).

In regards to human health, this may be an advantage due to the fact that increase in potassium intake has beneficial effects like reduced risk of high blood pressure, CVD, and stroke [59]. Additionally, high potassium diet lowers the risk of osteoporosis, renal disease, and kidney stones, in addition to preventing the progression of diabetes [60]. Similarly, there is evidence that high magnesium diet is inversely associated with the risk of type 2 diabetes [61], can lower blood pressure and the risk of CVDs [62]. Potato is not rich in calcium. Its decline due to the stress could even be positive (Table 4A), because excess oral Ca is a risk factor for CVDs in dialysis patients and can lead to aortic and coronary artery calcification, above all in patients with diabetes and chronic kidney disease (CKD) [63]. However, besides Ca, phosphorus should also be limited in patients with CKD, where the kidneys fail to excrete the mineral, causing disorders like vascular calcifications as frequently observed in CKD [64]. In this case, increase in P caused by DS as found in both years (Table 4B) is less optimal.

Table 4A. Contents of calcium and magnesium in tubers grown under control and drought stress conditions

Genotype	Calcium ($g\ kg^{-1}$)		Magnesium ($g\ kg^{-1}$)	
	Control	Stress	Control	Stress
Year (1)				
St 89403	0.33 ± 0.04a	0.30 ± 0a	0.83 ± 0.08a	0.93 ± 0.13a
St 3792	0.38 ± 0.04a	0.33 ± 0.04a	1.00 ± 0.12b	$1.15 \pm 0.11b^{*}$
Agave	0.35 ± 0.05a	0.30 ± 0a	0.88 ± 0.04ab	$1.05 \pm 0.05ab^{**}$
Average	0.35 ± 0 .05	$0.31 \pm 0.03^{*}$	0.90 ± 0.12	$1.04 \pm 0.14^{**}$
Year (2)				
St 89403	0.25 ± 0.05a	0.23 ± 0.04a	0.65 ± 0.05a	$0.73 \pm 0.04a^{*}$
St 3792	0.43 ± 0.04b	0.35 ± 0.11a	0.98 ± 0.18a	$0.98 \pm 0.11a^{I}$
Agave	0.40 ± 0.10b	0.25 ± 0.05a	0.73 ± 0.04a	$0.90 \pm 0a^{**I}$
Average	0.36 ± 0.10	$0.28 \pm 0.09^{*}$	0.78 ± 0.18^{H}	$0.87 \pm 0.13^{**H}$

a,b Genotype means followed by different letters in the same column differ significantly at $P \leq 0.05$. Difference between control and drought stressed tubers is significant at $^{*}P \leq 0.05$ and $^{**}P \leq 0.01$. Differences between the years are significant at $^{I}P \leq 0.05$ and $^{H}P \leq 0.01$.

Table 4B. Contents of phosphorus and potassium in tubers grown under control and drought stress conditions

Genotype	Phosphorus (g kg^{-1})		Potassium (g kg^{-1})	
	Control	Stress	Control	Stress
Year (1)				
St 89403	2.95 ± 0.11a	3.18 ± 0.18a	13.18 ± 0.68a	14.23 ± 0.29a*
St 3792	3.23 ± 0.08a	3.58 ± 0.19a**	14.53 ± 0.58a	15.78 ± 0.51b**
Agave	3.15 ± 0.15a	3.40 ± 0.16a	13.58 ± 1.04a	15.00 ± 0.21b*
Average	3.11 ± 0.17	3.39 ± 0.24**	13.76 ± 0.97	15.00 ± 0.73**
Year (2)				
St 89403	2.88 ± 0.13a	3.23 ± 0.19a*	11.48 ± 0.26a^{H}	13.95 ± 0.29a**
St 3792	3.03 ± 0.15a	3.28 ± 0.11a^{I**}	14.08 ± 1.93a	13.60 ± 1.11a
Agave	3.43 ± 0.16b	3.35 ± 0.22a	11.30 ± 0.29a^{I}	14.58 ± 0.12a*
Average	3.11 ± 0.28	3.28 ± 0.19*	12.28 ± 1.70^{I}	14.04 ± 0.78^{*I}

a,b Genotype means followed by different letters in the same column differ significantly at $P \leq 0.05$. Difference between control and drought stressed tubers is significant at $^{*}P \leq 0.05$ and $^{**}P \leq 0.01$. Differences between the years are significant at $^{I}P \leq 0.05$ and $^{H}P \leq 0.01$.

Free Amino Acids

In the frame of this study, 18 AAs were assayed in the control and drought stressed tubers. The results have shown that total amounts of free AAs were significantly increased due to DS (Table 5A), on average by 26.6% (1. year) and 16.9% (2. year).

This was observed for all GTs, except for St 89403 in the second test year. Above all asparagine (Asn) (Table 5A) and proline (Pro) (Table 5B) were elevated. The drought-induced raise in Asn ranged from 42.2% (2) to 49.3% (1), while Pro was comparable with +43.8% in the first year but was considerably higher (4-fold) under severe DS in the second year. These results were expected, as Pro is a stress related marker and mediates drought tolerance [65]. Moreover, gamma amino butyric acid (GABA), a non-protein amino acid was induced by the stress in every year (1. +7.6%; 2. +5.9%), while being less tremendously than in the case of proline (Table 5B). Nevertheless, GABA is also known to play a role in events from perception of environmental stresses to physiological responses [66] and mitigates stress.

In fact, free AAs and their derivatives serve as osmolytes which help maintain cell volume and stabilize proteins and other macromolecules, in order to adapt the cells to DS [67]. Interestingly, in the first year with moderate drought 17 of the 18 AAs tested in this work had increased values, while in the second test year with severe DS eight AAs were declined in their levels (Fig. 1). In this last case, it is possible that these AAs were partially incorporated into proteins and/or enzymes needed for adaptive responses and were thereby found to be reduced. On the other hand, it is also possible that a strong boost in Pro by severe DS (Table 5B) went on cost of the other AAs. Finally, the results may also demonstrate that the DS-induced changes in free AAs were dependent on the stress intensity (Fig. 1).

Table 5A. Total amounts of free amino acids and asparagine in tubers grown under control and drought stress conditions

Genotype	Free amino acids (g 100 g^{-1})		Asparagine (g 100 g^{-1})	
	Control	Stress	Control	Stress
Year (1)				
St 89403	1.34 ± 0.20a	1.52 ± 0.18a**	0.33 ± 0.08a	0.53 ± 0.09a**
St 3792	2.89 ± 0.36b	3.88 ± 0.30b*	1.00 ± 0.16b	1.52 ± 0.11b*
Agave	2.87 ± 0.20b	3.61 ± 0.16b*	0.91 ± 0.12b	1.31 ± 0.09b*
Average	2.37 ± 0.77	3.00 ± 1.08**	0.75 ± 0.32	1.12 ± 0.44***
Year (2)				
St 89403	1.71 ± 0.30a	1.39 ± 0.09a**	0.61 ± 0.13a^{I}	0.55 ± 0.05a
St 3792	3.44 ± 0.28b	4.63 ± 0.46b**	1.34 ± 0.18b	2.10 ± 0.28b^{**I}
Agave	3.54 ± 0.32b^{I}	4.16 ± 0.07b^{*H}	1.10 ± 0.10c^{I}	1.71 ± 0.02b^{**H}
Average	2.90 ± 0.89^{H}	3.39 ± 1.46^{*I}	1.02 ± 0.34^{H}	1.45 ± 0.68^{**H}

a,b,c Genotype means followed by different letters in the same column differ significantly at $P \leq 0.05$. Difference between control and drought stressed tubers is significant at $^{*}P \leq 0.05$, $^{**}P \leq 0.01$ and $^{***}P \leq 0.0001$. Differences between the years are significant at $^{I}P \leq 0.05$ and $^{H}P \leq 0.01$

With regards to human health, an increase in free amino acids is positive as they are used to synthesize proteins and other biomolecules when they are processed by the human body. For example, proline, an osmoprotectant in plants, also plays significant roles in human nutrition, protein biosynthesis, and metabolism, particularly in the synthesis of arginine, polyamines, and glutamate, in addition to wound healing processes and immune responses. Requirement of Pro for whole-body protein is seen as the greatest among all AAs [68]. Pro is a precursor for glutamate in the central nervous system (CNS) and considered to be a neurotransmitter [69, 70]. Impaired Pro metabolism has been implicated in a complex of neuropsychiatric disorders [69]. However, in patients with individual psychiatric disorders, elevated serum Pro levels can have negative effects on brain functions [71].

Table 5B. Contents of proline and GABA in tubers grown under control and drought stress conditions

Genotype	Proline (mg 100 g^{-1})		GABA (mg 100 g^{-1})	
	Control	Stress	Control	Stress
Year (1)				
St 89403	16.42 ± 3.85a	19.24 ± 1.07a	80.12 ± 5.16a	88.46 ± 1.91a*
St 3792	24.85 ± 2.73b	36.27 ± 7.27b*	223.15 ± 8.73b	230.64 ± 10.95b
Agave	25.97 ± 2.74b	41.17 ± 4.27b*	126.80 ± 1.38c	143.84 ± 4.58c*
Average	22.41 ± 5.30	32.22 ± 10.60**	143.36 ± 59.85	154.31 ± 58.92**
Year (2)				
St 89403	11.19 ± 3.46a	105.23 ± 21.03a^{**H}	116.42 ± 10.73a^{H}	83.06 ± 5.31a*
St 3792	27.11 ± 1.07b	54.98 ± 9.51b^{**I}	269.91 ± 5.14b^{H}	307.42 ±13.67b^{*HH}
Agave	32.51 ± 1.73c^{I}	125.78 ± 34.40a^{*H}	155.54 ± 13.59c^{I}	183.13 ± 10.77c^{*H}
Average	23.60 ± 9.34	95.33 ± 38.17^{***HH}	180.62 ± 65.95^{H}	191.20 ± 92.37^{H}

a,b,c Genotype means followed by different letters in the same column differ significantly at $P \leq 0.05$. Difference between control and drought stressed tubers is significant at $^{*}P \leq 0.05$, $^{**}P \leq 0.01$ and $P \leq 0.0001$. Differences between the years are significant at $^{I}P \leq 0.05$, $^{H}P \leq 0.01$ and $^{HH}P < 0.0001$.

Moreover, asparagine is needed for normal brain development and balances the CNS, *i.e.* Asn, which may prevent excess nervousness and anxiety [72]. GABA as a major inhibitory neurotransmitter in the CNS affects mood and activities, especially in stress situations and has the potential to alleviate the stress. Moreover, GABA is regarded to improve brain functions, *e.g.* memory and study capability, lower blood pressure, and may have relaxing effects in humans [73]. When orally administrated, GABA not only lowered anxiety but also elevated immunglobuline A (IgA) levels and enhanced immunity under stress conditions [74]. Lower GABA levels can be linked with psychiatric and neurological disorders such as increased sadness, anxiety, insomnia, depression, and epilepsy [75]. In summary, all this may reflect that individual stress-induced amino acids in plants are also effective in mitigating the impact of human stress. Plants and humans probably share similar strategies when it comes to coping with stress of varying intensities.

This study ascertained the effects of drought stress with different intensities on selected health relevant compounds in tuber tissue. The results revealed that DS has a clear effect on most quality parameters studied in this two-year experiment. For instance, glucose and fructose were significantly reduced in both years ($P < 0.05$ all), while sucrose increased, especially with severe drought in the second year ($P < 0.01$). TSS declined in the first year ($P < 0.01$), but were boosted in their tendency in the second year. Starch was also reduced in the first year ($P < 0.01$), but was less affected by severe DS in the second year. Crude proteins were significantly enhanced in both years ($P < 0.0001$, all). A similar clear increase was demonstrated for LAH and MI in every year ($P < 0.01$, all). Moreover, the portion of ALA on total FAs in the tubers was higher under stress ($P < 0.01$), while OLA, its precursor, declined ($P < 0.05$) but only in the first year with moderate stress. All other FAs were less affected by DS in every year. DS significantly increased the minerals Mg, K and P, whereas Ca was reduced ($P < 0.05$ both years) by the stress. Additionally, total amounts of free AAs were elevated in both years ($P < 0.05$ all). Above all, Asn and Pro ($P < 0.001$ both) in addition to GABA reached higher levels under DS conditions, especially in the first year ($P < 0.001$).

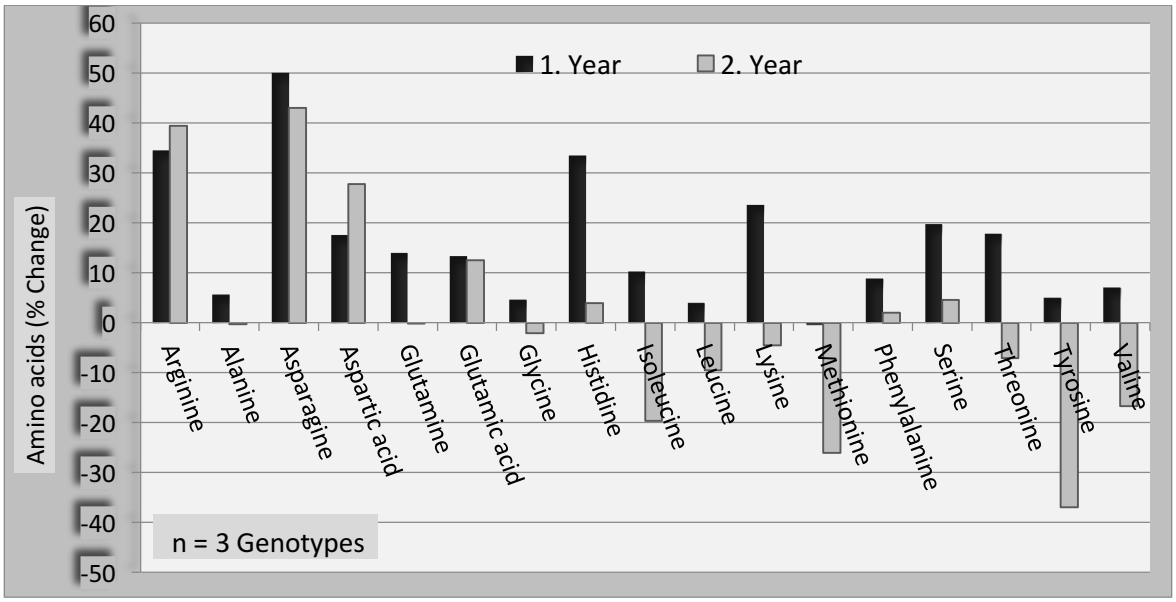

Figure 1. Percent change of free amino acids in tuber tissue under conditions of moderate (1. year) and severe drought stress (2. year) averaged over three GTs. Pro with +43.8% (1) and +303.9% (2) is not shown. All data of individual amino acids can be requested from the authors.

CONCLUSIONS

With regards to NCDs like type-2 diabetes, CVDs, and CKDs, it can be concluded that changes in nutritional and bioactive compounds caused by moderate and severe DS are beneficial and have the potential to improve the health quality of tubers. Nevertheless, this increase in quality is paid with a decrease of tuber yield.

List Of Abbreviations: Amino acids (AAs), abscisic acid (ABA), α-linolenic acid (ALA), asparagine (Asn), carbohydrates (CHO), cardiovascular diseases (CVDs), central nervous system (CNS), chronic kidney diseases (CKDs), cultivar (cv.), docosahexaenoic acid (DHA), drought stress (DS), dry matter (DM), eicosapentaenoic acid (EPA), essential amino acids index (EAAI), fatty acids (FAs), fatty acid methyl ester (FAME), fresh weight (FW), gamma aminobutyric acid (GABA), gas chromatography (GC), genotypes (GTs), glycemic index (GI), high performance liquid chromatography (HPLC), inductively coupled plasma optical emission spectroscopy (ICP-OES), inositol phospholipids (IP), inositol hexaphosphate (IP_6), jasmonic acid (JA), linoleic acid (LA), lipid acyl hydrolase (LAH), *myo*-inositol (MI), non-communicable diseases (NCDs), oleic acid (OLA), polyunsaturated fatty acids (PUFAs), proline (Pro), standard deviation (SD), total soluble sugars (TSS)

Competing interest: The authors have no financial interest or conflicts of interest.

Author's contribution: All authors contributed to this work

Acknowledgements: I. Schollenberg, M. Jennerjahn, M. Jugert and C. Leesch are thanked for excellent technical assistance. Additionally, we thank Dr. W. Sarich and A. Haack from the Agricultural Analysis and Research Institute (LUFA) Rostock, Germany for the analyses of minerals by ICP-OES.

REFERENCES

1. Farahani HA, Valadabadi SA, Daneshian J, Shiranirad AH, Khalvati MA: Medicinal and aromatic plants farming under drought conditions. J Hort Forest 2009, 6:86-92.
2. Rahdari P, Hoseini SM: Drought stress: A Review. Int J Agron Plant Prod. 2012, 3:443-446.
3. Passioura J: The drought environment: Physical, biological and agricultural perspectives. J Exp Bot 2007, 58:113-117.
4. Boyer JS: Plant productivity and environment. Science 1982, 218:443-448.
5. Alizadeh VV, Shokri A, Soltani, Yousefi MA: Effect of climate change and drought stress on plant physiology. Int J Adv Biol Biochem Res 2015, 3:38-42.
6. Shinozaki K, Yamaguchi-Shinozaki K: Gene networks involved in drought stress response and tolerance. J Exp Bot 2007, 58:221-227.
7. Kiribuchi K, Jikumaru Y, Kaku H, Minami E, Hasegava M, Kodama O *et al.*: Involvement of the basic helix-loop-helix transcription factor RERJ1 in wounding and drought stress responses in rice plants. Biosci Biotechnol Biochem 2005, 69:1042-1044.
8. Buckenhüskes HJ: Nutritionally relevant aspects of potatoes and potato constituents. In Potato in Progress: Science Meets Practice. Edited by Haverkort AJ, Struik PC. Wageningen:

Academic Publishers; 2005:17-26.
9. Nestares T, Lopez-Jurado M, Sanz A, Lopez-Frias M: Nutritional Assessment of two vegetable protein concentrates in rats. J Agric Food Chem 1993, 41:1282-1286.
10. Bártová V, Bárta J, Brabcová A, Zdráhal Z: Amino acid composition and nutritional value of four cultivated South American potato species. J Food Compos Anal 2015, 40:78-85.
11. Van Gelder WMJ, Vonk CR: Amino acid composition of coagulable protein from tubers of 34 potato varieties and its relationship with protein content. Potato Res 1980, 23:427-434.
12. Friedman M. Chemistry, biochemistry and dietary role of potato phenols: A Review. J Agric Food Chem 1997, 45:1523-1540.
13. Brown CR, Wrolstad R, Durst R,Yang CP, Clevidence B: Breeding studies in potatoes containing high concentrations of anthocyanins. Am J Pot Res 2003, 80:241-250.
14. Grace SC: Phenolics as antioxidants. In Antioxidants and Reactive Oxygen Species in Plants. Edited by Smirnoff N. Oxford: Blackwall Publishing Ltd; 2005:141-168.
15. Piironen V, Toivo J, Puupponen-Pimiä R, Lampi AM: Plant sterols in vegetables, fruits and berries. J Sci Food Agric 2003, 83:330-337.
16. Racette SB, Lin X, Lefevre M, Anderson-Spearie K, Most MM, Ma L, Ostlund RE: Dose effect of dietary phytosterols on cholesterol metabolism: A controlled feeding study. Am J Clin Nutr 2010, 91:32-38.
17. Jansen G, Flamme W, Schüler K, Vandrey M: Tuber and starch quality of wild and cultivated potato species and cultivars. Pot Res 2001, 44:137-146.
18. Glycemic Index Database: Potato. Edited by Burani J. Sydney: University of Sydney; 2015. http://www.glycemicindex.com/about.php.
19. Brand-Miller JC: Glycemic load and chronic disease. Nutr Rev 2003, 61:49-55.
20. Halton TL, Willett WC, Liu S, Manson JE, Stampfer MJ, Hu FB: Potato and french fry consumption and risk of type 2 diabetes in women. Am J Clin Nutr 2006, 83:284-290.
21. Khosravi-Boroujeni H, Mohammadifard N, Sarrafzadegan N, Sajjadi F, Maghroun M, Khosravi A *et al.*: Potato consumption and cardiovascular disease risk factors among Iranian population. Int J Food Sci Nutr 2012, 63:913-920.
22. Nugent AP: Health properties of resistant starch. British Nutrition Foundation. Nutr Bulletin 2005, 30:27-54.
23. Lockyer S, Nugent AP: Health effect of resistant starch. British Nutrition Foundation. Nutr Bulletin 2017, 1-32.
24. Raatz SK, Idso L, Johnson LK, Jackson MI, Combs GF: Resistant starch analysis of commonly consumed potatoes: Content varies by cooking method and service temperature but not by variety. Food Chem 2016, 208:297-300.
25. Katona P, Katona-Apte J: The interaction between nutrition and infection. Clin Infect Dis 2008, 46:1582-1588.
26. Singh MB, Lakkshminarayana J, Fotedar R, Anand PK: Childhood illnesses and malnutrition in under five children in drought affected desert area of Western Rajastan, India. J Commun Dis 2006, 38:88-96.
27. Wegener CB, Jansen G, Jürgens HU: Bioactive compounds in potatoes: Accumulation under drought stress conditions. Funct Food Health Dis 2015, 5:108-116.
28. Niederer IB, Manzardo GG, Amado R: A reinvestigation of the derivatization of monosaccharides as aldonitrile peracetates. Carbohyd Res 1995, 278:181-194.
29. Ewers E: Determination of the starch content by a polarimetric method. Z Öffentl Chem 1908,

14:150-157.
30. Kjeldahl J: New method for the determination of nitrogen in organic matter. Z Anal Chem 1883, 22:366-382.
31. Bohac RJ: A modified method to purify patatin from potato tuber. J Agric Food Chem 1991, 39:1411-1415.
32. Wegener CB, Jansen G, Jürgens HU: Influence of drought and wounding stress on soluble phenols and proteins in potato tubers. Sust Agric Res 2014, 3:1-15.
33. Bligh EG, Dyer WJ: A rapid method of total lipid extraction and purification. Can J Biochem Physiol 1959, 37:911-917.
34. Arens M, Schulte E, Weber K: Fatty acid methylesters, transesterifikation with trimethyl sulfonium hydroxide (Rapid method). Fat Sci Technol 1994, 96:67-68.
35. Müller M, Arnold-Fußhöller H, Bischoff M, Ellinghaus R, Janssen E, Leiterer M *et al.*: Analysis of individual elements in plant material and feedstuff by ICP-OES. In Book of Methods III. ICP-OES Feedstuff, 10.8.2. Darmstadt: VDLUFA Publishers; 2006:1-12.
36. Cohen SA, Michaud DP: Synthesis of a fluorescent derivatizing reagent, 6-amino-quinolyl-N-hydroxysuccinimidyl carbamate, and its application for the analysis of amino acids via high performance liquid chromatography. Anal Biochem 1993, 211:279-287.
37. Hernández-Orte P, Ibarz MJ, Cacho J, Ferreira V: Amino acid determination in grape juices and wines by HPLC using a modification of the 6-amino-quinolyl-N-hydroxy-succinimidyl carbamate (AQC) method. Chromatographia 2003, 58:29-35.
38. Van Dam RM, Seidell JC: Carbohydrate intake and obesity. Eur J Clin Nutr 2007, 61: 75-99.
39. Foster-Powell K, Holt SHA, Brand-Miller JC: International table of glycemic index and glycemic load values. Am J Clin Nutr 2002, 76:5-56.
40. Lister CE, Munro J: Nutrition and health qualities of potatoes - A future focus. In Crop & Food Research Confidential Report. Christchurch: New Zealand Institute of Crop & Food Research Ltd.; 2000, 143:8-9.
41. Liu YW, Han CH, Lee MH, Hsu FL, Hou WC: Patatin, the tuber storage protein of potato (*Solanum tuberosum* L.), exhibits antioxidant activity in vitro. J Agric Food Chem 2003, 51:4389-4393.
42. Racusen D: Lipid acyl hydrolase of patatin. Can J Bot 1984, 62:1640-1644.
43. Galliard T: The enzymatic deacylation of phospholipids and galactolipids in plants. Biochem J 1971, 121:379-390.
44. Verma DC, Dougall DK: Biosynthesis of *myo*-inositol and its role as a precursor of cell-wall polysaccharides in suspension cultures of wild-carrot cells. Planta 1979, 146:55-62.
45. Stevenson JM, Perera IY, Heilmann I, Persson S, Boss WF: Inositol signaling and plant growth. Trends Plant Sci 2000, 5:252-257.
46. Cantley LC: Phosphoinositol Signaling and Disease. Proceedings of the International Conference; Inositol Phospholipid Signaling in Physiology and Disease. 26-27 June 2012; New York. Edited by: The New York Academy of Science; 2012, 1.
47. Holub BJ: The nutritional significance, metabolism, and function of *myo*-inositol and phosphatidylinositol in health and disease. Adv Nutr Res 1982, 4:107-141.
48. Constantino D, Minozzi G, Minozzi F, Guaraldi C: Metabolic and hormonal effects of *myo*-inositol in women with polycystic ovary syndrome: a double blind trial. Eur Rev Med Pharmacol Sci 2009, 13:105-110.
49. Dinicola S, Chiu TTY, Unfer V, Carlomagno G, Bizzarri M: The rationale of the *myo*-inositol

and D-chiro-inositol combined treatment for polycystric ovary syndrome. J Clin Pharmacol 2014, 54:1079-1092.

50. Fruzzetti F, Perini D, Russo M, Bucci F, Gadducci A: Comparison of two insulin sensitizers, metformin and *myo*-inositol, in women with polycystic ovary syndrome (PCOS). Gynecol Endocrinol 2016, 3:1-4.
51. Levine J, Barak Y, Gonzalves M, Szor H, Elizur A, Kofman O, Belmaker RM: Double-blind, controlled trial of inositol treatment of depression. Am J Psychiatry 1995, 5:792-794.
52. Vucenik I, Shamsuddin AM: Cancer inhibition by inositol hexaphosphate (IP_6) and Inositol: From laboratory to clinic. J Nutr 2003, 133:3778-3784.
53. Kärenlampi SO, White PJ: Potato proteins, lipids, and minerals. In Advances in Potato Chemistry and Technology. Edited by Sing J, Kaur L. Burlington: Elsevier Inc.; 2009, 99-125.
54. Schaller F, Schaller A, Stintzi A: Biosynthesis and metabolism of jasmonates. J Plant Growth Regul 2005, 23:179-199.
55. Heldt HW: Glycerolipids are important membrane building stones. In Plant Biochemistry, 3 Edition. Edited by Heldt HW. Heidelberg, Berlin: Spectrum Academic Publisher; 2003, 15:372-375.
56. Burdge GC: Metabolism of α-linolenic acid in humans. Prostag Leucotr Ess 2006, 75: 161-168.
57. Swanson D, Block R, Mousa SA: Omega-3 fatty acids EPA and DHA: Health benefits throughout life. Adv Nutr 2012, 3:1-7.
58. Rao TSS, Asha MR, Rao KSJ: Understanding nutrition, depression and mental illness. Indian J Psychiatry 2008, 50:77-82.
59. Haddy FJ, Vanhoutte PM, Feletou M: Role of potassium in regulating blood flow and pressure. Am J Physiol Regul Integr Comp Physiol 2006, 290:546-552.
60. He FJ, McGregor GA. Beneficial effects of potassium on human health. Physiol Plantarum 2008, 133:725-735.
61. Dong J, Xun P, He K, Quin L: Magnesium intake and risk of type 2 diabetes. Diabetes Care 2011, 34:2116-2122.
62. Chaudhary DP, Sharma R, Bansal DD: Implication of magnesium deficiency in type 2 diabetes: A Review. Biol Trace Elem Res 2010, 134:119-129.
63. Andress DL: Bone and mineral guidelines for patients with chronic kidney disease: A call for revision. Clin J Am Soc Nephrol 2008, 3:179-183.
64. Fourtounas C: Phosphorus metabolism in chronic kidney disease. Hippokratia 2011, 15:50-52.
65. Munawarti A, Taryono I, Semiarti E, Holford P, Sismindari S: Tolerance of accessions of Glagah (*Saccharum Spontaneum*) to drought stress and their accumulation of proline. Am J Agric Biol Sci 2013, 8: 1-11.
66. Kinnersley AM, Turano FJ: Gamma amino butyric acid and plant responses to stress. Crit Rev Plant Sci. 2000, 19:479-509.
67. Yancey PH: Water stress, osmolytes and proteins. Amer Zool 2001, 42:699-709.
68. Wu G, Bazer FW, Burghardt RC, Johnson GA, Kim SW, Knabe DA *et al.*: Proline and hydroxyproline metabolism: Implications for animal and human nutrition. Amino Acids 2011, 40:1053-1063.
69. Hu CA, Phang MJ, Valle D: Proline metabolism in health and disease. Amino Acids. 2008,

35:651-652.

70. Günes M, Bulut M, Demir S, Ibiloglu AO, Kaya MC, Atli A *et al.*: Dignostic performance of increased prolidase activity in schizophrenia. Neurosci Lett 2016, 613:36-40.
71. Vorstman JAS, Turetsky BI, Kemner C: Proline affects brain function in 22q11 DS children with the low activity $COMT^{158}$ allele. Neuropsychopharmacol 2009, 34:739-746.
72. Amino acid (protein) deficiency signs. In Nutrient and Health/Disease Associations Report 2016: 1-11. http://www.healingwithnutrition.com/aminoacid.html.
73. Yoto A, Murao S, Motoki M, Yokoyama Y, Horie N, Takeshima K *et al.*: Oral intake of gamma-amino butyric acid affects mood and activities of the central nervous system during stressed conditions induced by mental tasks. Amino Acids 2012, 43:1331-1337.
74. Abdou AM, Higashiguchi S, Horie K, Kim M, Hatta H, Yokogoshi H: Relaxation and immunity effects of gamma-aminobutyric acid (GABA) administration in humans. BioFactors 2006, 26:201-208.
75. Gamma-aminobutyric acid (GABA). Alternative Medicine Review 2007, 12:274-279.