Nutritional value and antioxidant activity of sprouts from seeds of *Carica* papaya – their benefits for broiler nutrition

Wike Winarti, Turrini Yudiarti, Endang Widiastuti, Hanny Indrat Wahyuni, Tri Agus Sartono and Sugiharto Sugiharto*

Universitas Diponegoro, Faculty of Animal and Agricultural Sciences, Semarang, Central Java, Indonesia (50275) *Corresponding author: sgh_undip@yahoo.co.id

Abstract

Winarti, W., Yudiarti, T., Widiastuti, E., Wahuni, H., Sartoro, T. A. & Sugiharto, S. (2024). Nutritional value and antioxidant activity of sprouts from seeds of *Carica papaya* – their benefits for broiler nutrition. *Bulg. J. Agric. Sci.*, *30*(1), 107–114

The impact of germination on nutritional and antioxidant properties of papaya seeds was evaluated. Germination began with soaking of dried seeds and germinating at room temperature for 15 days. The experiment were designed as a 2×2 factorial (papaya varieties and germination) with four replicates. Total germination was higher in Bangkok than in California. Crude protein and moisture contents were higher, while crude fat, total energy and energy from fat were lower in Bangkok than its counterpart. Total unsaturated fatty acids (FA) and n-3 polyunsaturated FA were higher in California than in Bangkok. Amino acids (L-serine, L-alanine, L-lysine, L-tyrosine, L-proline and L-threonine) differed between Bangkok and California. Antioxidant activity was higher in California. Sprouting increased crude protein, crude fibre and moisture, while decreasing ash, crude fat, total energy and energy from fat. Germination decreased total saturated FA, while increasing n-3 and n-6 polyunsaturated FA. Sprouting increased antioxidant activity and amino acid L-phenylalanine and L-threonine, L-alanine, L-aspartate, L-proline, L-serine and L-tyrosine. In conclusion, Bangkok variety showed better germination indexes, higher crude protein and lower crude fat and energy than those of California. California papaya had higher unsaturated FA and n-3 polyunsaturated FA. Sprouting increased crude protein, crude fibre, moisture, n-3 and n-6 polyunsaturated FA, amino acids and antioxidant activity, while decreasing ash, crude FA.

Keywords: antioxidant; amino acids; fatty acids; germination; papaya seed; variety

Introduction

Broiler chickens are today a very important livestock commodity for satisfying the animal protein needs of the Indonesian population. Broiler chicks are typically raised in stressful conditions, such as microclimates outside of their comfort zones, overcrowding, poor hygiene, and poor rearing management. As a result of these conditions, broiler chickens are often stressed leading to the impaired production and health. To alleviate the negative effects of these unpleasant conditions, farmers commonly employ synthetic antioxidants in drinking water or feed. Yet, the excessive usage of synthetic antioxidants may leave residues in broiler meat, putting consumer's health at risk (Sugiharto, 2019). For this reason, natural antioxidants may be a viable option for overcoming stress and increasing broiler chicken productivity and health (Dassidi et al., 2020).

Papaya (*Carica papaya* L.) is a tropical fruit that is readily accessible all year (Ameen et al., 2012). In Indonesia, the production of papaya is increasing year after year. According to data of the Statistics Indonesia (BPS, 2019), papaya production was 986 992 tons in 2010 and expanded to 1 016 388 tons in 2020 (BPS, 2020), with an average consumption of 8.38 grams per person per day in 2020 (BPS, 2019). Bangkok papaya and California papaya are two varieties of papaya that are now widely cultivated by Indonesian farmers. These varieties are popular among consumers because of their soft texture and sweet taste. Apart from the flesh, papaya seeds are part of the papaya fruit that cannot be consumed by humans. Indeed, the papaya seeds made up 16% of the papaya weight (Malacrida et al., 2011). Owing to this fact, the rise in papaya fruit production and consumption corresponds to an increase in papaya seed waste (Lestari et al., 2018), which, if not handled and utilized appropriately, may pose environmental issues.

In addition to having a high protein content (25.63%) (Malacrida et al., 2011), papaya seeds are abundant in polyphenolic compounds, flavonoids, saponins, and tannins, all of which can serve as natural sources of antioxidants for broiler chickens (Sugiharto, 2020). Despite the numerous beneficial active components, papaya seeds contain a high crude fiber content (45.6%) (Maisarah et al., 2014), as well as anti-nutritional compounds that can decrease the rate of digestion and absorption of nutrients, thus reducing broiler growth. In light of these circumstances, efforts should be made to improve the nutritional content of papaya seeds in order to optimize their use by broiler chicks.

Germination or sprouting is a simple technique for enhancing the nutritional and functional values of seeds (Sugiharto, 2021). Martinez et al. (2013) showed that the nutritional quality of soybean sprouts was improving, notably their total protein content, which was higher than that of non-germinated seeds. The germination process has also been linked to higher levels of protein, vitamins, calcium, magnesium, iron, zinc, phosphorus, and potassium of mungbean. Moreover, total amino acids (valine, tryptophan, histidine, isoleucine, leucine, methionine, phenylalanine, lysine and threonine) in mungbean also increased with germination (Elobuike et al., 2021). Sprouting, on the other hand, reduced various antinutritional components in mungbean, such as phytate, oxalate, trypsin inhibitor, and tannin (Elobuike et al., 2021). Indeed, the nutrient content in seeds becomes unbound (free) during germination, which can help to improve digestibility (Ferdiawan et al., 2019). In term of functional property, Tarasevičienė et al. (2019) reported that germination increased the antioxidant activity of seeds. They also discovered that following germination broccoli, radish, and alfalfa seeds had higher total phenol concentration.

Based on this prior research, germination appears to be a simple and effective strategy for improving the nutritional content and increasing antioxidant properties of papaya seeds. As a result, employing papaya seed sprouts as a natural antioxidant source in broiler chickens could be a viable alternative to synthetic antioxidants. The aim of this study was to compare the germination performance and to examine the impact of germination on the nutritional value and antioxidant capacity of Bangkok and California papaya seeds.

Materials and Methods

Germination test and sprouting experiment

The seeds of Bangkok and California papayas were collected from ripe papaya fruits, which has a yellowish green skin colour in Bangkok papaya and a reddish yellow skin colour in California papaya. The seeds were separated from the flesh, rinsed and then sun-dried. To measure the percentage of total germination, average germination time, and time to achieve 50% germination, a germination test was conducted, according to Falcinelli et al. (2020), with four replicates (100 seeds in each replicate). The germination test began with the soaking of dried papaya seeds in water for 7 hours before sowing. Papaya seeds used for germination were seeds that sink during soaking. The selected seeds were then germinated in plastic trays containing sterile paper laid over sterile cotton wetted with water. A perforated cap was placed over the trays to allow air circulation while preventing dehydration. The germination was maintained at room temperature (±28°C), in a light: dark regime of 12:12 hours. Every day after the third day of incubation, each tray was sprayed with 5 mL of water. The number of seeds that germinated was recorded daily for 15 days. Sprouts were considered "ready-toeat" between the completion of cotyledon enlargement and just prior to the emergence of the first genuine leaf.

Chemical analysis

The proximate contents of germinated seeds were determined according to the standard AOAC method (AOAC, 1995). The amino acid contents were measured based on the standard ultra-performance liquid chromatography (UPLC) method (Szkudzinska et al., 2017). The antioxidant activity of germinated seeds was determined according to the 2.2-diphenylpicrylhydrazyl (DPPH) free radical scavenging assay as described by Wu et al. (2009) with few modifications. The total phenolic content of germinated papaya seeds was determined using the Folin-Ciocalteu method, which was modified slightly from Orak (2006). According to Ghasemzadeh et al. (2010), a spectrophotometric technique with aluminum chloride was used to determine the concentration of flavonoids in germinated papaya seeds. Using an oxygen bomb calorimeter (Parr Instruments Co., Moline, IL, USA), the gross energy content in germinated papaya seeds was measured. As a calibration standard, benzoic acid was used. The fatty acid content of germinated papaya seeds was determined using gas chromatography. By comparing the retention times of each sample to the standard retention times, fatty acids were determined. The area percentage was normalized and converted to mg per 100 g of edible portion using a lipid conversion factor for fatty acid quantification (Holland et al., 1998).

Statistical analysis

Student's T-test was used to statistically analyze data on total germination, mean germination time, and time to reach 50% germination. On the basis of a 2×2 factorial design, the other data were processed using General Linear Models Procedure (SAS Inst. Inc., Cary, NC, USA). The data are presented as means and standard error.

Results and Discussion

Germination indexes of papaya seeds

Total germination was higher (p < 0.05) in Bangkok variety than that in California variety (Table 1). This finding was in accordance with Das et al. (2014) revealing the variation in seed germination between Sunrise Solo and Washington papaya cultivars. In this case, the difference in the sexual reproductive type and genetic variation between two varieties seemed to be responsible for the different total germination of papaya seeds (Brown et al., 2012). While mean germination

Table 1.	Germination	indexes of	papaya	seeds
----------	-------------	------------	--------	-------

Items	Bangkok	California	SE	p value
	variety	variety		
G (%)	93.3ª	71.8 ^b	3.70	0.02
MGT (DAS)	6.00	6.25	0.08	0.35
TG50 (DAS)	6.75 ^b	12.8ª	0.85	< 0.01

^{a,b}Means within the same row marked by superscript letters differ substantially (p < 0.05)

G: total germination, MGT: mean germination time, TG50: time to reach 50% germination, DAS: days after sowing, SE: standard error of the means

Table 2. Chemical compositions of papaya seeds

time was not different (p > 0.05) between two papaya varieties, the time to reach 50% germination was higher (p < 0.05) in California than that in Bangkok variety. In Maradol papaya variety, (Hernández & Tapia-Vargas, 2019) reported that the germination response period ranged from 9 to 14 days, with the peak germination occurring at 12 days. Regarding the time to reach 50% germination, to date no literature on papaya seed has been found to compare our finding with other study.

Overall, Falcinelli et al. (2020) suggested that the seed germination indexes are affected by ripening stages of fruits (in association with the maturity of embryo), the presence of germination inhibitor (abscisic acid) and germination conditions (such as light, hydration and temperature). In this study, both papaya seeds came from ripe papaya, and the germination conditions were kept the same. Regarding the presence of germination inhibitors, we did not determine such compounds in the present study.

Chemical compositions of papaya seeds

Table 2 presents the nutritional characteristics of papaya seeds. There was no notable interaction (p > 0.05) between papaya varieties and germination with regard to the nutritional characteristics of papaya seeds. It was observed in this study that crude protein and moisture contents were higher (p < 0.05), while crude fat, total energy and energy from fat were lower (p < 0.05) in Bangkok papaya than those in California papaya. In agreement, Santos et al. (2014) and Yanty et al. (2014) showed the differences in proximate composition among the varieties of papaya seeds. In addition to natural variation among varieties, the differences in proximate content between varieties of papaya seeds can also be due to the climatic conditions, growing seasons and cultivation sites (Nwofia et al., 2012). Irrespective of papaya varieties, sprouting was associated with the increase (p < 0.05) in crude protein, crude fibre and moisture, while decreasing (p < 0.05) ash, crude fat, total energy and energy from fat.

14	Baı	ngkok	Cal	ifornia	OE.	p value		
Items	Seed	Sprout Seed		Sprout	SE	Var	Germ	Var*Germ
Crude protein (% DM)	25.6 ^{bx}	27.8 ^{ax}	24.5 ^{by}	26.16 ^{ay}	0.32	< 0.01	< 0.01	0.35
Crude fibre (% DM)	36.0 ^b	46.0ª	33.7 ^b	35.25 ^b	1.06	0.06	0.01	0.05
Moisture (% DM)	8.88 ^{bx}	10.3 ^{ax}	7.23 ^{by}	9.53 ^{ay}	0.35	0.79	< 0.01	0.36
Ash (% DM)	6.52ª	5.89 ^b	6.45ª	5.56 ^b	0.14	0.19	< 0.01	0.55
Crude fat (% DM)	25.9 ^{ay}	22.5 ^{by}	30.9 ^{ax}	25.29 ^{bx}	1.52	0.03	0.01	0.49
Carbohydrates (% DM)	33.2	33.5	30.9	33.46	0.70	0.44	0.34	0.45
Total energy (kcal/100 g)	468 ^{ay}	448 ^{by}	500 ^{ax}	466 ^{bx}	6.31	0.01	0.01	0.46
Energy from fat (kcal/100 g)	233ªy	203 ^{by}	278 ^{ax}	228 ^{bx}	0.01	0.03	0.01	0.49

^{a,b} Means between seed and sprout marked by superscript letters differ substantially (p < 0.05)

^{xy}Means between Bangkok and California variety marked by superscript letters differ substantially (P < 0.05)

DM: dry matter, Var: papaya varieties (Bangkok and California), Germ: germinated or not, SE: standard error of the means

The increase in protein content of papaya seed following sprouting process in this study was in agreement with Devi et al. (2015) revealing the increase (by 9–12%) in crude protein content in cowpea (*Vigna unguiculata*) following germination at 25°C for 24 h. During germination process, some metabolic enzymes such as proteinases are activated resulting in the release of certain amino acids and peptides, as well as the synthesis or use of these amino acids and peptides to produce new proteins. As a result, sprouting increased the content of proteins in seeds (Devi et al., 2015; Benincasa et al., 2019). In accordance with Devi et al. (2015), the apparent increase in protein content in the sprouted seeds in this study could also be due to the loss of dry matter (i.e., crude fat) during sprouting through respiration.

The increase in crude fibre was observed in the sprouted papaya seeds as compared to that in non-sprouted papaya seeds. In line with our finding, Megat et al. (2016) reported that germination resulted in increased total fibre contents in Kidneys beans, Mung beans, soybeans and peanuts following the sprouting process. Also, Devi et al. (2015) reported an increase in crude fibre content in cowpea after germination. While Devi et al. (2015) suggested that the increase in fibre content of sprouted seeds was only as apparent and attributed to the reduced dry matter during germination, Megat et al. (2016) and Benincasa et al. (2019) revealed that formation of new polysaccharides (primary cell walls) during germination may be responsible for the increased total fibre in the sprouted seeds. It was shown in the present study that moisture content of papaya seeds increased with sprouting process, which was in line with Devi et al. (2015). They showed an increase in moisture content of cowpea after sprouting. In general, seeds absorb water (imbibitions) during the sprouting process, which thereby increased moisture content of sprouted seeds.

Sprouting was associated with the reduced (p < 0.05) ash content of papaya seeds. This result was in contrast to that reported by Devi et al. (2015), who found that sprouting of cowpeas increased their ash content. Yet, our result was in accordance with Atlaw and Kumar (2018) who reported a decreased ash content in fenugreek seed with germination. They further suggested that the decrease in ash content was attributable to the leaching of minerals during steeping (prior to sowing). It was apparent in this present study that sprouting resulted in decreased (p < 0.05) crude fat in papaya seeds. This result was in accordance with that of documented by Devi et al. (2015) and Atlaw & Kumar (2018). Devi et al. (2015) suggested that the decrease in fat content was related to the depletion of stored fat, which contributed to the seeds' catabolic processes during sprouting. Lipids, as the reserved nutrient, may be degraded during sprouting to give

the energy needed for protein synthesis and plant growth. Different from Devi et al. (2015) who showed a decrease in carbohydrate content in cowpea during sprouting, result in the present study did not show any change (p > 0.05) in carbohydrate content with germination. To date, the exact reason for these divergence remains unknown. In most cases, the utilization of carbohydrate as a readily available energy source during sprouting has been linked to a decrease in carbohydrate during sprouting (Atlaw & Kumar, 2018).

In this study, the use of fat, rather than carbohydrate, as an energy source seemed to save carbohydrate in the papaya seeds during germination process. In contrast to our study, Zhao et al. (2018) documented that the fat content in Chloris virgata, Kochia scoparia, Lespedeza hedysaroides, Astragalus adsurgens, Leonurus artemisia, and Dracocephalum moldavica remained constant during germination, while starch/ carbohydrate content decreased with germination. The rate of breakdown of fat and carbohydrate in seed as an energy source during the germination process may be determined by the variation in seed species, energy reserve (whether starchy or fatty seeds), and condition during sprouting such as oxygen concentration, temperature, steeping process, etc. (Atlaw & Kumar, 2018; Zhao et al., 2018). Total energy and energy from fat decreased (p < 0.05) with germination process in this present study. Corresponding results were reported by Masood et al. (2014), in which sprouting decreased energy values of mung bean and chickpea seeds. In this study, the decrease in total energy and energy from fat seemed to be attributed to the reduced fat content of papaya seeds during the germination process.

Fatty acids profile of papaya seeds

Data in the present study showed that total UFA and n-3 PUFA varied (p < 0.05) with the papaya varieties (Table 3). These data were in accordance with that of reported by Yanty et al. (2014) and Hssaini et al. (2020) in which fatty acids profile of seeds differed among the varieties of papaya. In addition to varieties, the variation in fatty acid profile of papaya seeds was also attributed to growing location, climatic and soil conditions as well as maturity stage of the fruits (Hssaini et al., 2020). In this study, germination resulted in decrease (p < 0.05) in total SFA, had no substantial effect on total UFA, while increasing (p < 0.05) the concentrations of n-3 PUFA and n-6 PUFA of papaya seeds. In agreement with our study, Shirvani et al. (2016) reported that germination reduced the content of SFA in safflower seed. According to Benincasa et al. (2019), the free fatty acids are broken down during germination via the β -oxidation and glyoxylate cycles, which were then converted into sugars. This may consequently reduce the content of SFA while increasing the carbohydrate content

Items	Ban	gkok	California			p value			
	Seed	Sprout	Seed	Sprout	SE	Var	Germ	Var*Germ	
Total SFA (g/100 g)	6.26ª	4.39 ^b	6.32ª	5.15 ^b	0.28	0.36	< 0.01	0.43	
Total UFA (g/100 g)	19.6 ^b	18.2 ^b	24.6ª	20.1ª	0.86	0.02	0.05	0.28	
n-3 PUFA (mg/100 g)	1.01 ^{by}	1.26 ^{ay}	1.32 ^{bx}	1.57 ^{ax}	< 0.01	< 0.01	< 0.01	1.00	
n-6 PUFA (mg/100 g)	0.30 ^b	0.50ª	0.40 ^b	0.60ª	0.09	0.10	< 0.01	1.00	

Table 3. Fatty acids profile of papaya seeds

^{a,b} Means between seed and sprout marked by superscript letters differ substantially (p < 0.05)

^{xy}Means between Bangkok and California variety marked by superscript letters differ substantially (P < 0.05)

SFA: saturated fatty acids, UFA: unsaturated fatty acids, PUFA: polyunsaturated fatty acids, Var: papaya varieties (Bangkok and California), Germ: germinated or not, SE: standard error of the means

in sprouts. In agreement with our present finding, Shirvani et al. (2016) revealed that PUFA increased in germinated seeds when compared with that in raw seeds. More specific, germination increased the content of C18:3 in safflower. Indeed, there is a formation of PUFA during germination process that may consequently increase the content of PUFA in sprouts (Kouamé et al., 2018). The n-3 and n-6 PUFAs are important structural cell components that can affect membrane fluidity and permeability, as well as the activity of membrane-associated enzymes, when they are incorporated in membrane phospholipids. In this respect, the increase of PUFA during germination may be essential and subjected to sustain the growth of sprouts (Doria et al., 2019).

Amino acid profile of papaya seeds

Table 4 showed the amino acid profile of sprouts of papaya seeds. It was apparent that some amino acids, including L-serine, L-alanine, L-lysine, L-tyrosine, L-proline and L-threonine, differed (p < 0.05) between Bangkok and California papaya varieties. This finding was in line with that of previously revealed by Dakare et al. (2011) and Sugiharto (2020) that different varieties may be attributed to the different amino acid composition of papaya seeds. In addition to varieties, Oyeleke et al. (2017) documented that the variation of amino acid profile in papaya seeds among the cultivars are dependent on the maturation/ripening stages of papaya. Irrespective of the different cultivars, sprouting was associated with the increase in some essential amino acids in papaya seeds, including L-phenylalanine and L-threonine, and non-essential amino acids such as L-alanine, L-aspartate, L-proline, L-serine and L-tyrosine. In line with our finding, former studies also reported that sprouting increased some essential and non-essential amino acids in lentil cultivars (Sulieman et al., 2008) and broccoli seeds (Tarasevičienė et al., 2009). The changes in amino acids during germination has been attributed to storage protein hydrolysis (by proteolytic enzymes into peptides and amino acids after 2-3 days from imbibition), re-arrangement

and synthesis of new amino acids to support the growth of sprouts (Tarasevičienė et al., 2009; Benincasa et al., 2019). In contrast to the above mentioned amino acids, the content of L-glutamic acid in papaya seed decreased with germination process. This result was in agreement with Choi et al. (2009) showing the decreased in L-glutamic acid in rice 'Keunnun' and 'Ilpumbyeo' with germination process. Also, Sulieman et al. (2008) showed the decreased glutamic acid in lentil cultivar with sprouting process. The decrease in glutamic acid in sprouts of papaya seeds seemed to be associated with the α -decarboxylation of L-glutamic acid (catalysed by glutamate decarboxylase) to produce γ -Aminobutyric acid (GABA) during the germination process (Benincasa et al., 2019).

Antioxidant properties of papaya seeds

There was significant interaction between varieties and germination with regard to antioxidant activity and the content of polyphenols and flavonoid in the sprouts of papaya seed (Table 5). Antioxidant activity was higher (p < 0.05) in California papaya sprouts compared to Bangkok papaya sprouts and non-germinated papaya seeds. In this study, sprouting significantly increased the antioxidant activity of papaya seeds. This was in line with the findings of Fouad et al. (2015), who found that the antioxidant activity (DPPH radical scavenging percent) in lentil seeds rose as the sprouting process progressed. There are several explanations on why the antioxidant activity increase with germination. Benincasa et al. (2019) suggested that germination may increase the activity of endogenous hydrolytic enzymes that hydrolyse the bound fraction of some compounds responsible for antioxidant activity in plant-based materials. Such hydrolysis may result in higher free or unbound antioxidants in sprouts. Moreover, Fouad et al. (2015) revealed that the increase in vitamin C and tocopherol formation during the sprouting process could be attributable to the enhanced antioxidant activity in sprouts. Yet, the increased antioxidant activity in sprouts of Bangkok and California papaya seemed

I4	Ban	gkok	Calif	ornia	SE		p value	
Items	Seed	Sprout	Seed	Sprout	SE	Var	Germ	Var*Germ
	·	H	Essential amin	no acids				
L-Histidine	4.26	4.44	3.79	4.47	0.19	0.60	0.31	0.54
L-Isoleucine	5.91	6.14	5.89	5.85	0.09	0.41	0.66	0.48
L-Leucine	8.10	8.64	8.39	8.50	0.12	0.77	0.21	0.40
L-Lysine	23.8 ^x	25.7 ^x	19.8 ^y	20.7 ^y	0.73	< 0.01	0.16	0.57
L-Phenylalanine	7.27 ^b	8.94ª	7.59 ^b	8.71ª	0.30	0.93	0.02	0.62
L-Threonine	12.2 ^{bx}	13.8 ^{ax}	10.4 ^{by}	12.3ªy	0.36	< 0.01	< 0.01	0.66
L-Valine	5.49	5.52	5.95	5.92	0.12	0.10	0.99	0.91
		No	n-essential ar	nino acids				
L-Alanine	3.79 ^d	5.04 ^b	4.40°	7.88ª	0.40	< 0.01	< 0.01	< 0.01
L-Arginine	18.2	16.1	19.7	18.0	0.67	0.22	0.17	0.86
L-Aspartate	22.4 ^b	27.7ª	21.5 ^b	26.0ª	0.79	0.21	< 0.01	0.71
L-Glutamic acid	28.8ª	25.3 ^b	29.8ª	24.1 ^b	0.81	0.92	< 0.01	0.39
L-Glycine	6.18	6.11	6.30	6.56	0.14	0.39	0.77	0.61
L-Proline	30.6 ^{bx}	32.4 ^{ax}	23.6 ^{by}	25.9 ^{ay}	0.92	< 0.01	< 0.01	0.45
L-Serine	10.9 ^{bx}	12.3 ^{ax}	9.22 ^{by}	11.2 ^{ay}	0.32	< 0.01	< 0.01	0.42
L-Tyrosine	35.0 ^{bx}	40.7 ^{ax}	23.4 ^{by}	30.7 ^{ay}	1.84	< 0.01	< 0.01	0.69

Table 4. Amino acid profile of papaya seeds

a,b,c,d Means within the row of L-Alanine marked by superscript letters differ substantially (p < 0.05)

^{a,b} Means between seed and sprout marked by superscript letters differ substantially (p < 0.05)

^{x,y} Means between Bangkok and California variety marked by superscript letters differ substantially (p < 0.05)

DM: dry matter, Var: papaya varieties (Bangkok and California), Germ: germinated or not, SE: standard error of the means

Table 5. Antioxidant properties of papaya seeds

Items	Bang	gkok	Calif	ornia	SE	p value		
Items	Seed	Sprout	Seed	Sprout	Sprout		Germ	Var*Germ
Antioxidant activity (IC_{50}^{\ddagger}) (ppm)	6399ª	3921°	4684 ^b	2998 ^d	0.03	< 0.01	< 0.01	0.01
Polyphenols (mg/g)	1.43ª	0.95°	1.28 ^b	0.98°	0.05	0.11	< 0.01	0.03
Flavonoids (µg/g)	683ª	392 ^b	402 ^b	388 ^b	0.02	< 0.01	< 0.01	< 0.01

 ${}^{a,b,c,d}\mbox{Means}$ within the same row marked by superscript letters differ substantially (p < 0.05)

 $^{\ddagger}IC_{50}$ is the effective concentration at which the 2,2-diphenylpicrylhydrazyl (DPPH) radicals were scavenged by 50%. A lower IC50 value is associated with a stronger DPPH radical scavenging activity.

Var: papaya varieties (Bangkok and California), Germ: germinated or not, SE: standard error of the means

not to be contributed by the polyphenols and flavonoids contents in sprouts.

This latter inference was actually supported by Falcinelli et al. (2020) reporting that besides phenols, other antioxidants may contribute to the antioxidant activity of sprouts from citrus seeds. In contrast to the antioxidant activity, the contents of polyphenols were lower (p < 0.05) in sprouts of Bangkok and California papaya than that in raw seeds. With regard to flavonoids, germination reduced (p < 0.05) flavonoids contents in Bangkok papaya, but had no notable effect on the flavonoid content in California papaya. The reduce in polyphenols and flavonoids contents in papaya sprouts were contrary to Falcinelli et al. (2020) who documented that sprouting increased the content of total phenols and phenolic acids in citrus seeds. The disparities between our results and those of the other authors were most likely attributable to genotype differences and sprouting conditions (e.g., temperature, germination time, and biotic and abiotic stress during sprouting) (Chon, 2013; Falcinelli et al., 2020).

Conclusion

Bangkok variety had better germination indexes, higher crude protein and lower crude fat and energy than those of California papaya. Total UFA and n-3 PUFA were higher in California than that in Bangkok variety. The variety of papaya also determine amino acids profile of seeds. Irrespective of papaya variety, sprouting increased crude protein, crude fibre and moisture, while decreasing ash, crude fat and energy. Germination decreased total SFA, while increasing n-3 PUFA and n-6 PUFA concentrations in papaya seeds. Sprouting also increased essential and non-essential amino acids and antioxidant activity of papaya seeds. Overall, sprouting could be a simple and cheap method to improve the nutritional and functional properties of papaya seeds, and thereby beneficial for broiler nutrition.

References

- Ameen, S. A., Adedeji, O. S., Ojedapo, L. O., Salihu, T. & Fakorede, O. L. (2012). Anthemintic efficacy of pawpaw (*Carica papaya*) seeds in commercial layers. *African Biotechnology*, 11(1), 126-134.
- **AOAC** (1995). Official methods of analysis of the Association of Analytical Chemists. OAOC International, Washington, DC.
- Atlaw, T. K. & Kumar, J. Y. (2018). Effect of germination on nutritional composition and functional properties of fenugreek (*Trigonella foenum-graecum* Linn) seed flour. *Food Science* and Quality Management, 76, 18-23.
- Statistics Indonesia (BPS) (2019). Fruit Consumption in Indonesia in 2019. Jakarta: Statistics Indonesia.
- Statistics Indonesia (BPS) (2019). Fruit Crop Production in 2019. Jakarta: Statistics Indonesia.
- **Statistics Indonesia (BPS)** (2020). Fruit Crop Production in 2020. Jakarta: Statistics Indonesia.
- Benincasa, P., Falcinelli, B., Utts, S., Stagnari, F. & Galieni, A. (2019). Sprouted grains: a comprehensive review. *Nutrients*, *11*(2), 1-29.
- Brown, J. E., Bauman, J. E., Lawrie, J. F., Rocha, O. J. & Moore, R. C. (2012). The structure of morphological and genetic diversity in natural populations of *Carica papaya* (*caricaceae*) in Costa Rica. *Biotropica*, 44(2), 179-188.
- Choi, I., Suh, S. J., Kim, J. H. & Kim, S. L. (2009). Effects of germination on fatty acid and free amino acid profiles of brown rice 'keunnun'. *Food Science and Biotechnology*, 18(3), 799-802.
- Chon, S. U. (2013). Total polyphenols and bioactivity of seeds and sprouts in several legumes. *Current Pharmaceutical Design*, 19(34), 6112-6124.
- Dakare, M. A., Ameh, D. A. & Agbaji, A. S. (2011). Biochemical assessment of 'Daddawa' food seasoning produced by fermentation of pawpaw (*Carica papaya*) seeds. *Pakistan Journal of Nutrition*, 10(3), 220-223.
- Das, S. C., Dinesh, M. R., Das, A. & Suresh, C. P. (2014). Studies on fruit set and germination in some papaya cultivars. *Acta Horticultura*, 10(22), 87-89.
- Dassidi, N., Oumbortime, N., Kouame, Y. A. E., Kokou, V., Decuypere, E., Gbeassor, M., Onagbesan, O. & Tona, K. (2020). Effect of pawpaw (*Carica papaya*) seed diets on production performance of boiler breeders and hatching parameter. *International Journal of Poultry Science*, 19(1), 1-9.
- Devi, V. B., Kshwaha, A. & Kumar, A. (2015). Sprouting characteristics and associated changes in nutritional composition of cowpea (*Vigna unguiculata*). Food Science and Technology, 52(10), 6821-6827.
- Doria, E., Pagano, A., Ferreri, C., Larocca, A. V., Macovei, A.,

Araujo, S. D. S. & Balestrazzi, A. (2019). How does the seed pre germinative metabolism fight against imbibition damage? Emerging roles of fatty acid cohort and antioxidant defense. *Frontiers in Plant Science, 10*(1505), 1-13.

- Elobuike, C. S., Idowu, M. A., Adeola, A. A. & Bakare, H. A. (2021). Nutritional and functional attributes of mungbean (*Vi-gna radiata* [L] Wilczek) flour as affected by sprouting time. *Legume Science*, 3(4), 1-11.
- Falcinelli, B., Famiani, F., Paoletti, A., D'Egidio, S., Stagnari, F., Galieni, A. & Benincasa, P. (2020). Phenolic compounds and antioxidant activity of sprouts from seeds of citrus species. *Agriculture*, 10(33), 1-9.
- Ferdiawan, N., Nurwantoro, N. & Dwiloka, B. (2019). The effect of germination time on the physical and chemical properties of Tolo bean flour (*Vigna unguiculata* L). *Journal of Food Technology*, 3(2), 349–354.
- Fouad, A. A. & Rehab, F. M. A. (2015). Effect of germination time on proximate analysis, bioactive compounds and antioxidant activity of lentil (*Lens culinaris medik.*) sprouts. *Acta Scientiarum Polonorum, Technologia Alimentaria*, 14(3), 233-246.
- Ghasemzadeh, A., Jaafar, H. Z. E. & Rahmat, A. (2010). Antioxidant activities, total phenolics and flavonoids content in two varieties of Malaysia young ginger (*Zingiber officinale* Roscoe). *Molecules*, 15(6), 4324–4333.
- Hernández, J. C. Á. & Tapia-Vargas, L. M. (2019). Selection of outstanding papaya plants in commercial environments for breeding purposes. *Revista Mexicana de Ciencias Agrícolas*, 10(23), 303-311.
- Holland, B., Welch, A. A., Unwin, I. D., Buss, D. H., Paul, A.
 A. & Southgate, D. A. T. (1998). The composition of foods. London: The Royal Society of Chemistry.
- Hssaini, L., Hanine, H., Charafi, J., Razouk, R., Elantari, A., Ennahli, S., Alvares, F. & Ouaabou, R. (2020). First report on fatty acids composition, total phenolics and antioxidants activity in seeds oil of four fig cultivars (*Ficus carica L.*) grow in Morocco. *Oilseeds and Fats Crops and Lipid*, 27(8), 3-10.
- Kouamé, D. A. I., Faulet, B. W., Ekissi, G. S. E., Fagbohoun, B. J. & Kouame, P. L. (2018). Effects of sprouting time on physicochemical and biochemical parameters of oil extracted from sesame (*Sesamum indicum* L.) Seeds grown in Côte d'Ivoire. *International Journal of Current Research*, 10(6), 70358-70364.
- Lestari, A. R. A., Syahfitri, S. A., Cahyo, S. T., Wardaniati, I. & Herli, M. A. (2018). Antibacterial activity of papaya seed infusion (*Carica papaya* L.) on *Escherichia coli*, Salmonella thypi and Staphlycocus aureus. Pharmacy and Science, 1(2), 39-45.
- Maisarah, A., Asmah, M. R. & Fauziah, O. (2014). Proximate analysis, antioxidant and anti-proliferative activities of different parts of *Carica papaya*. Journal of Tissue Science and Engineering, 5(1), 1-7.
- Malacrida, C. R., Kimura, M. & Jorge, N. (2011). Characterization of a high oleic oil extracted from papaya (*Carica papaya* L.) seeds. *Ciência e Tecnologia de Alimentos*, 31(4), 929–934.
- Martínez, M., Díaz, M. F., Hernández, Y. & Sierra, S. F. (2013). Replacement of commercial soybean (*Glycine max*) by germinated and non-germinated raw grain meal in broiler diets. *Live*stock Research for Rural Development, 25(7), 1-10.

- Masood, T., Shah, U. & Zeb, A. (2014). Effect of sprouting time on proximate composition and ascorbic acid level of mung bean (*Vigna radiate* L.) and chickpea (*Cicer arietinum* L.) seeds. *Journal of Animal and Plant Science*, 24(3), 850-859.
- Megat, R. M. R., Azrina, A. & Norhaizan, M. E. (2016). Effect of germination on total dietary fibre and total sugar in selected legumes. *International Food Research Journal*, 23(1), 257-261.
- Nwofia, G. E., Ojimelukwe, P. & Eji, C. (2012). Chemical composition of leaves, fruit pulp and seeds in some *Carica papaya* (L) morphotypes. *International Journal of Medicinal and Aromatic Plants*, 2(1), 200-206.
- **Orak, H. H.** (2006). Total antioxidant activities, phenolics, anthocyanin, polyphenoloxidase activities and its correlation of some important red wine grape varieties, which are grown in Turkey. *EJPAU*, *9*(18), 235-241.
- Oyeleke, G. O., Adetoro, R. O., Sulaiman, W. K. & Adebisi, A. A. (2017). Amino acid and functional characteristics of pawpaw (*Carica papaya*) seeds under normal storage ripening. *Advance Research*, 10(2), 1-6.
- Santos, C. M. D., Abreu, C. M. P. D., Freire, J. M., Queiroz, E. D. R. & Mendonca, M. M. (2014). Chemical characterization of the flour of peel and seed from two papaya cultivars. *Food Science and Technology*, 34(2), 353-357.
- Shirvani, A., Jafari, M., Goli, S. A. H., Tehrani, N. S. & Rahimmalek, M. (2016). The changes in proximate composition, antioxidant activity and fatty acid profile of germinating safflower (*Carthamus tinctorius*) seed. *Journal of Agricultural Science* and Technology, 18, 1967-1974.
- Sugiharto, S. (2020). Papaya (*Carica papaya* L.) seed as a potent functional feedstuff for poultry – A review. *Veterinary World*, 13(8), 1613–1619.
- Sugiharto, S. (2021). The use of sprouted grains as dietary feed ingredients for broilers-a brief overview. *Livestock Research for*

Rural Development, 33(3), 1-6.

- Sugiharto, S., Yudiarti, T., Isroli, I., Widiastuti, E., Wahyuni, H. I. & Sartono, T. A. (2019). Fermented feed as a potential source of natural antioxidants for broiler chickens-a mini review. Agriculturae Conspectus Scientificus, 84(4), 313-318.
- Sulieman, M. A., Eltayeb, M. M. & Babiker, E. E. (2008). Effect of sprouting on chemical composition and amino acid content of Sudanese lentil cultivar. *Journal of Applied Science*, 8(12), 2337-2340.
- Szkudzinska, K., Smutniak, I., Rubaj, J., Korol, W. & Bielecka, G. (2017). Method validation for determination of amino acids in feed by UPLC. *Accreditation and Quality Assurance*, 22, 247–52.
- Tarasevičienė, Ž., Danilčenko, H., Jarienė, E., Paulauskienė, A.
 & Gajewski, M. (2009). Changes in some chemical components during germination of broccoli seeds. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*, 37(2), 173-176.
- Tarasevičienė, Ž., Viršilė, A., Danilčenko, H., Duchovskis, P., Paulauskienė, A. & Gajewski, M. (2019). Effects of germination time on the antioxidant properties of edible seeds. *Cy-TA-Journal of Food*, 17(1), 447-454.
- Wu, N., Fu, K., Fu, Y. J., Zu, Y. G., Chang, F. R., Chen, Y. H., Liu, X. L., Kong, Y., Liu, W. & Gu, C. B. (2009). Antioxidant activities of extracts and main components of pigeonpea (*Caja-nus cajan* (L.) Millsp.) leaves. *Molecules*, 14(3), 1032-1043.
- Yanty, N. A. M., Marikkar, J. M. N., Nusantoro, B. P., Long, K. & Ghazali, H. M. (2014). Changes in some chemical components during germination of broccoli seeds. *Journal of Oleo Science*, 63(9), 885-892.
- Zhao, M., Zhang, H., Yan, H., Qiu, L. & Baskin, C. C. (2108). Mobilization and role of starch protein, and fat reserve during seed germination of six wild grassland species. *Frontiers in Plant Science*, 8(9), 1-11.

Received: August, 10, 2022; Approved: November, 20, 2022; Published: February, 2024