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Seed Quality, and Fatty Acid and Sugar Contents of Pepper Seeds (*Capsicum annuum* L.) in Relation to Seed Development and Drying Temperatures

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Abstract: This study was carried out in order to determine the effects of drying temperature (25, 35, and 45 °C) and the developmental stage (55, 65, 75, and 85 days after anthesis (DAA)) on germination, vigor, and the sugar (sucrose, glucose, fructose) and fatty acid contents of pepper seeds (*Capsicum annuum* L.) over 2 consecutive years. In seeds harvested at 75 DAA and after, seed viability and vigor were not influenced by drying at temperatures up to 45 °C. Linoleic acid (18:2) was the main fatty acid in pepper seeds, comprising 75-80% of total fatty acids. It was followed by oleic (18:1) and palmitic (16:0) as roughly 10%-12% and stearic (18:0) as 3%. The results indicated that drying seeds at different temperatures does not change fatty acid composition. Approximately 30% of the pepper seeds are constituted of sucrose, and this did not change with maturity level between 55 and 85 DAA, nor did it change at different drying temperatures. However, the levels of fructose and glucose gradually decreased as the developmental stage advanced and both were lower than 4% of total sugar at the final harvest. Seeds dried at 45 °C had lower amounts of sugar compared to those dried at 25 and 35 °C. Seed quality and fatty acid and sucrose composition do not change as long as the seeds are harvested within 75 DAA.

Key Words: Pepper, seed drying, seed development

Biber Tohumlarının (*Capsicum annuum* L.) Kalite, Yağ Asitleri ve Şeker Kapsamının Tohum Gelişimi ve Kurutma Sıcaklığına Bağlı Olarak Değişimi

Özet: Bu çalışmada, biber tohumlarının (*Capsicum annuum* L.) tohum çimlenmesi ve tohum gücü ile toplam yağ asitleri ve şeker içeriği (sukroz, glikoz, fruktoz) üzerine, farklı gelişme devrelerinin (55, 65, 75 ve 85 DAA-Çiçeklenmeden Sonraki Gün Sayısı) ve kurutma sıcaklıklarının (25, 35 ve 45 °C) etkisi belirlenmiştir. Elde edilen sonuçlara göre, tohumların çiçeklenmeden sonra 75. gün ve sonrası hasat edilmeleri şartıyla 45 °C'ye kadar çıkan sıcaklıklarda kurutulmaları halinde tohum kalitesinde (canlılık ve güç) olumsuz bir etkiye rastlanmamıştır. Biber tohumlarında toplam yağ asitlerinin % 75-80'ini linoleik asit (18:2) oluşturmuştur. Bunu yaklaşık % 10-12 ile oleik (18:1) ve palmitik (16:0) asitler ve de % 3 ile stearik (18:0) asit izlemektedir. Çalışmada, farklı sıcaklıklarda kurutulmuş tohumların yağ asitleri içeriği benzer bulunmuştur. Fruktoz ve glikoz gelişme dönemi ilerledikçe azalmaya başlamakta ve son hasatta toplam şekerin % 4'ünden de düşük seviyeye inmektedir. Her iki yılda da, 45 °C'de kurutulan tohumların şeker kapsamı, 25 ve 35 °C'de kurutulan tohumlardan daha düşük seviyede olmuştur. Sonuç olarak, biberde tohumlar çiçeklenmeden sonra 75. günde hasat edilmek kaydıyla, 25 ve 45 °C arasındaki kurutma sıcaklıkları tohum kalitesi, yağ asidi ve sukroz bileşimini etkilememektedir.

Anahtar Sözcükler: Biber, tohum gelişimi, tohum kurutma

Introduction

Desiccation of orthodox seeds on the mother plant is a part of the seed development program that allows them to survive in subsequent dry storage. Those seeds that went through natural desiccation drying on the mother plant such as soya bean, rape seed, cotton, pea, and bean seeds dry to very low moisture contents, e.g., 10%, on the plant. However, in fleshy fruited species such as

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pepper, in which seeds do not undergo natural desiccation drying on the plant, seed moisture remain considerably high at maturation, e.g., 40% (Demir and Ellis, 1992). Seeds in fleshy fruited species, the same as most orthodox crop species, are dried to around 10% of their fresh weight since metabolic activities have almost stopped, pathogen activity is minimal, and the seeds are able to be stored. It was reported that the drying temperature and the developmental stage are factors affecting the quality and potential longevity of seeds following drying (Sinniah et al., 1998). This may be rather important in fleshy fruited crops such as pepper since the magnitude of reduction in seed moisture content during drying is great, from about 40%-45% to 7%-8%. The rate of moisture loss depends on both the drying temperature and the ambient relative humidity. Moreover, a mixture of seeds at various developmental stages within the same lot due to the continuously flowering nature of pepper plants might also be an influential response to drying conditions.

The response to dehydration is associated with various biochemical changes in the seeds. Sugars (e.g., sucrose) are known to protect both membranes (Sinniah et al., 1998) and the cytoplasm (Corbineau et al., 2000) to increase the acquisition of dehydration tolerance of the seeds. Moreover, storage of fatty acids that accumulate during seed development may be an important target of peroxidative attack during drying, in turn reducing the seed quality (Corbineau et al., 2000).

This study was carried out in order to determine the effect of drying temperature and developmental stage on changes in germination and quality, as well as the levels of sucrose and other sugars and fatty acid composition of pepper seeds.

Materials and Methods

Plant culture

Pepper plants (*Capsicum annuum* L.) of the cultivar 'Sera Demre' were grown in open-field conditions in the growing seasons of 2002 and under glasshouses in 2003 at the Experimental Field at the Faculty of Agriculture, University of Ankara, and West Mediterranean Agricultural Research Institute, Antalya, Turkey, respectively. Plant spacing was 75 cm between plants and 40 cm between rows. One month after transplanting and during the seed filling phase (20-25 days after anthesis) 15 kg of ammonium sulfate and 15 kg of potassium nitrate were applied per 1000 m^2. Three hundred flowers were tagged at full anthesis in the first and second flowering layers of the plants in each year and fruits were harvested at 55, 65, 75, and 85 days after anthesis (DAA). Roughly 75 fruits were harvested at each harvest time. Fruits were selected randomly among the flowers that were tagged.

Seed drying

Seeds were extracted by hand following harvest and 15 g of fresh seeds in each harvest were stored immediately after harvest (within 20 min) at -80 °C for lipid and sugar content analyses. The remaining seeds were dried at 25, 35, and 45 °C at 35% relative humidity, which was achieved with a saturated $CaCl_2$ solution on top of the mesh trays in closed containers until 10% for 48 h. The final seed moisture content following drying was determined.

Seed dry mass and moisture determinations

The seed dry mass per harvest was calculated following drying 3 replicate batches of 50 seeds at 130 °C for 2 h. Seeds were weighed after cooling in a desiccator containing silica gel and dry weight was expressed as mg $seed^{-1}$. The seed moisture content (fresh weight basis) was determined according to ISTA rules (low temperature oven method, 105 °C, 17 h) (ISTA, 1996).

Germination and vigor tests

Seed germination tests after harvest (fresh seed germination) and after drying (dry seed germination) were carried out in 3 replicates of 50 seeds per harvest at 25 °C for 14 days (ISTA, 1996). Fifty seeds were placed on 2 layers of Whatman No. 1 paper wetted with 5 cm^3 of distilled water in 9 cm diameter petri dishes. Then they were put into plastic bags in order to prevent water loss and placed in an incubator in the dark. A 2 mm length of radicle was used as the germination criterion. In order to determine changes in seed vigor, a modified (modification was to temperature only, not to ageing period or ageing box) accelerated ageing test was conducted according to the procedure described by Hampton and TeKrony (1995). Distilled water (40 cm^3) was added to each plastic box (11 × 11 × 4 cm) and 150 seeds were put on a wire mesh tray (10 × 10 × 3 cm) and placed in the box. Seeds were aged at 45 °C for 72 h. Following the ageing process, seeds were removed from the box and kept at room temperature for 2 h. Then a standard germination test (50 × 3 replicates) was conducted at 25 °C for 14 days in the dark.

Seed sugar content determination

Seeds were removed from storage at -80 °C and the sucrose, fructose, and glucose were extracted. The extraction procedure was in accordance with Sinniah et al. (1998) with slight modifications. One gram of seeds was used in 5 cm^3 of 80% (v/v) ethanol using a mortar and pestle. The suspension was moved from the mortar into a plastic centrifuge tube and incubated in a water bath at 80 °C for 15 min. The homogenate was centrifuged at 3000 rpm for 10 min. The pellet was then washed with 2 cm^3 of 80% ethanol, incubated at 80 °C for 15 min, and recentrifuged. The supernatants were combined and reduced to dryness using a rotary evaporator at 40 °C. The residue was taken up in 8 cm^3 of nanopure water and filtered through a 0.45 µm filter. A 20 µl aliquot was subjected to high performance liquid chromatography analyses. The separation of sugars was achieved by a RCM-Monosaccharides column (Phenomenex Ltd, Cheshire, UK) heated to 80 °C. The mobile phase was 5 mM H_2SO_4. Sugars were identified by comparison of retention times with known standards (Sigma). Quantification was achieved by integration of elution peak areas and comparison with known amounts of external standards.

Seed fatty acid determination

Lipid contents of fresh and dried seeds were determined using Soxhlet extraction and hexane was used as solvent (IUPAC, 1987). Oil samples were esterified with 2 N methanol - KOH solution (IUPAC, 1987) and fatty acid methyl esters were analyzed using a gas chromatograph (Thermo Quest Trace 2000, Milan, Italy) equipped with a fused silica capillary column BPX 70 (30 m × 0.25 µm ID and 0.25 mm film thickness) (SGE Int., Pty, Ltd., Victoria, Australia) The split ratio was 80:1 and the carrier gas was helium at 1.0 ml min^{-1}. Injector, column, and detector temperatures were 230, 190, and 240 °C, respectively.

Data analyses

The data were analyzed by ANOVA and significance level was determined as 5% using SPSS. Angular transformation was applied for germination values before analyses.

Results

Seed moisture content varied from 45% to 59%, and dry seed mass from 4.1 to 4.8 mg $seed^{-1}$ among harvests in pepper seeds (Table 1). The end of the seed-filling phase and the time of maximum dry mass (mass maturity) during development occurred in the first harvest of the experiment, 55 days after anthesis (DAA), and subsequently did not change ($P > 0.05$) (Table 1).

Seeds harvested at 55 DAA and dried at 25, 35, and 45 °C had significantly the lowest germination percentages compared to the other harvests in the first year. However, 65 DAA and after, neither drying nor harvest affected germination percentages, which varied between 92% and 100%. In the second year, germination percentages of dried seeds were not affected by any factors except at 45 °C and the results were between 90% and 99% (Table 2). Seeds harvested between 65 and 85 DAA had similar vigor values and germination after AA in both years. However, seeds harvested at 55 DAA were of lower quality than those of the other harvests.

Table 1. Seed moisture content (%), seed weight (mg $seed^{-1}$), and fresh seed germination percentage of pepper seeds harvested 55, 65, 75, and 85 days after anthesis (DAA) in 2002 and 2003.

Year	Harvest	Seed moisture content (%)	Seed weight (mg)	Fresh seed germination (%)
2002	55	59	4.4 a	71 b
	65	53	4.7 a	93 a
	75	52	4.3 a	91 a
	85	52	4.8 a	94 a
2003	55	47	4.3 a	93 b
	65	48	4.6 a	89 c
	75	48	4.2 a	99 a
	85	45	4.7 a	97 a

* Means with different letters in the same column and the year are significantly different at 5% level.

Table 2. Interactive effects of pepper seeds harvested 55, 65, 75, and 85 DAA and dried at 25, 35, and 45 °C on germination percentage (GP) and vigor (germination after accelerated ageing, AA) in 2002 and 2003.

		Drying Temperatures (°C)								
		25		35		45				
Year	Harvest	GP	AA	GP	AA	GP	AA	H	T	H × T
2002	55	93	26	91	35	89	23	GP : *	ns	ns
	65	99	60	98	72	100	63	AA : *	*	*
	75	98	59	99	71	98	83			
	85	97	59	96	71	94	65			
2003	55	99	51	94	38	90	55	GP : ns	*	*
	65	98	53	95	36	92	66	AA : *	ns	*
	75	98	63	90	69	98	70			
	85	96	74	92	72	96	77			

H: Harvest, T: Temperature, GP: Germination percentage,
AA: Accelerated ageing test, ns: Not significant, *: significant ($P < 0.05$)

Linoleic acid (18:2) was the most abundant fatty acid and constituted the maximum amount in total lipid content in both fresh and dried pepper seeds. In addition, between 77% and 82% and between 72% and 76% of total lipid content of fresh pepper seeds were composed of linoleic acid in the 2 years, respectively (Table 3).

Oleic and palmitic acid contents of pepper seeds showed very similar values. Oleic acid content changed between 6.4% and 13.6% in fresh and between 9.3% and 10.7% in dried seeds in 2002. Corresponding values in 2003 were between 10.9% and 12.1% in fresh and between 9.5% and 10.7% in dried seeds. Drying reduced the percentage of oleic acid content in both years except in one harvest (55 DAA, 2002). Approximately 10% of total fatty acid was composed of palmitic acid in pepper seeds, which was nearly the same between fresh and dry seeds.

Fresh pepper seeds harvested at 55 DAA showed reasonably reduced levels of sugars (glucose and fructose). The content of fructose declined substantially just after 10 days (65 DAA) and remained stable thereafter, while glucose content decreased rather gradually. At the final harvest, both had less than 10 mg g^{-1} dry weight. Sucrose content did not change with drying temperature when seeds were harvested at 85 DAA. It was at the highest level in seeds harvested between 65 and 75 DAA, and dried at 25 °C (Figure).

Discussion

The high seed moisture content values (45%-59%) observed in this study show a typical trend in various fleshy fruited species at maturity (Welbaum and Bradford, 1989; Demir and Ellis, 1992). The deposition of storage substances is one of the key processes of zygotic embryogenesis. The time of the occurrence of maximum seed dry weight (maximum storage reservation) during development in this study (55 DAA) was in accordance with findings of previous studies in pepper (Demir and Ellis, 1992; Demir, 2002), reported as 49-53 DAA.

Seed germination percentages after harvest (fresh seeds) before drying reached a maximum by 65 DAA in the first year and 75 DAA in the second year and remained stable thereafter (Table 1). Positive effects of drying in early harvest, 55 DAA (compare Tables 1 and 2), were also observed in castor bean (*Ricinus communis*) and aubergine seeds in early stages of development (Kermode and Bewley, 1989). It is likely to be effective in shifting seeds from developmental to germination mode (Kermode and Bewley, 1989). Moreover, combined high moisture content of seeds and temperature at the beginning of drying might be copying the development of the mother plant and therefore advancing the germination of early harvests of these peppers. Such effects were also reported in immature muskmelon seeds by Welbaum and Bradford (1989).

Table 3. Changes in fatty acid composition (%) of pepper seeds in relation to seed harvest (55, 65, 75, and 85 DAA) and drying at 25, 35, and 45 °C in 2002 and 2003.

				Drying Temperatures (°C)		
Year	Harvest	Fatty acids	Fresh seeds	25	35	45
2002	55	Linoleic	82.0	83.0	82.0	82.0
		Oleic	6.40	10.1	9.80	10.7
		Palmitic	7.40	10.3	10.4	10.6
		Stearic	4.10	3.70	3.10	3.10
	65	Linoleic	79.0	81.0	76.0	82.0
		Oleic	12.6	9.60	10.2	9.30
		Palmitic	13.6	10.4	10.1	10.7
		Stearic	3.80	3.10	2.90	2.90
	75	Linoleic	77.0	81.0	81.0	81.0
		Oleic	13.1	9.80	9.70	9.80
		Palmitic	14.5	10.3	10.2	10.4
		Stearic	4.10	3.60	3.40	3.10
	85	Linoleic	79.0	69.0	79.0	79.0
		Oleic	21.6	9.30	10.1	9.70
		Palmitic	12.6	12.2	10.6	10.1
		Stearic	4.50	2.90	3.70	3.20
2003	55	Linoleic	74.0	74.0	74.0	74.0
		Oleic	10.9	10.3	10.3	10.1
		Palmitic	11.7	11.7	11.8	11.7
		Stearic	3.00	3.30	3.30	3.10
	65	Linoleic	72.0	74.0	74.0	74.0
		Oleic	11.0	9.90	10.7	10.4
		Palmitic	11.6	12.0	11.1	11.7
		Stearic	4.50	2.90	3.00	3.00
	75	Linoleic	76.0	74.0	69.0	72.0
		Oleic	12.1	9.90	9.50	9.90
		Palmitic	10.2	12.0	11.3	13.8
		Stearic	4.20	3.20	4.00	4.00
	85	Linoleic	74.0	75.0	75.0	75.0
		Oleic	12.1	9.60	9.50	9.60
		Palmitic	9.20	11.3	11.5	11.4
		Stearic	3.20	2.90	2.90	3.10

The lowest level of quality was in the first harvest (55 DAA) and the highest one was 85 DAA in 2002 (Table 2). This shows that resistance to high temperature drying, which might be considered a quality component, increases after seeds attained the maximum dry mass for some 10 days or more. This conclusion was in agreement with previous findings of a number of studies on seed development that maximum seed germination and vigor occur some time after attainment of maximum seed dry mass in a number of crop species (Welbaum and Bradford, 1989; Demir and Ellis, 1992).

Our results are consistent with Xu and Kafkafi's (2003) findings on the fatty acid profile of mature pepper seeds. The most abundant fatty acid in mature pepper seeds was linoleic acid (62%-67%), followed by oleic (15%-18%), palmitic (13%-15%), and stearic (3%-4%) acids.

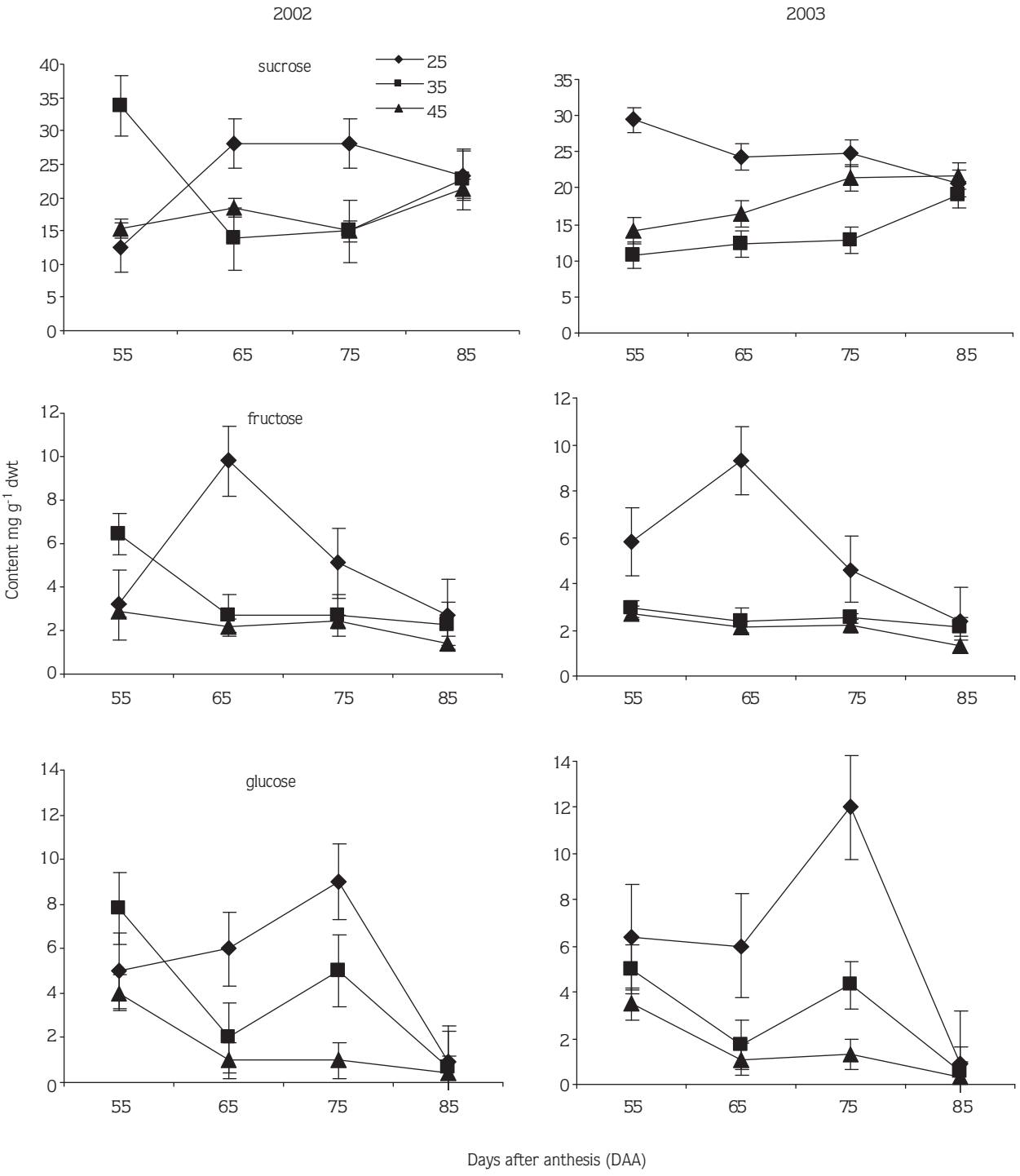

Figure. Changes in sucrose (mg g^{-1}, s), glucose (mg g^{-1}, f), and fructose (mg g^{-1}, g) contents of pepper seeds dried at 25 (♦), 35 (■), and 45 (▲) °C during development (55, 65, 75, and 85 DAA) in 2002 and 2003. Measurements were repeated twice. SE of means shown as error bars.

High glasshouse temperature during seed development resulted in a decrease in linoleic acid content. Martiner-Force et al. (1998) indicated that temperature affects seed fatty acid composition mainly by altering the linoleic acid ratio (unsaturated) in sunflower seeds. Wang et al. (2000) found that increased stearic acid percentage can be detrimental to the viability and quality of soybean seeds. They assumed that this may be due to the effect on the physical properties of triaglycerols and membrane phospholipids. Accumulation of stearate in membrane lipids also made membranes less adaptable to changes in temperature and moisture content (Thompson and Li, 1997). However, the stearic acid content of pepper seeds (2.9%-4.5%) is much lower than that of soybeans (9.6%-11.6%). Moreover, its content was slightly reduced or unchanged by drying.

It was reported that sucrose may serve as an agent of the desiccation tolerance mechanism (Corbineau et al., 2000). Data obtained from immature wheat (Black et al., 1999) and embryonic axis of pea (Corbineau et al., 2000) suggest that oligosaccharide biosynthesis is regulated by the rate of water loss. In our study sucrose content did not change remarkably among seeds dried at different temperatures compared to that of fresh ones. This contrasting conclusion may be due to the differences in the developmental stages in various studies.

Pepper seeds completed dry weight accumulation and the seed filling phase by the first harvest (55 DAA). However, harvests in previous studies started at much earlier stages of development (e.g., 35 DAA, at the beginning of the seed filling stage). Therefore, dehydration tolerance was found to be correlated as seeds accumulate sucrose during development. Sinniah et al. (1998) argued that differences in seed quality among different commercial seed lots of *Brassica campestris* (rapa) L. originate from sugars as well as differences in accumulation of heat-stable proteins. Moreover, Chen and Burris (1990) suggest that changes in soluble sugar composition especially the ratio of raffinose to sucrose rather than the absolute content of sugars was highly correlated with membrane stability during high temperature drying.

Seed vigor is one of the main traits of seed quality that is under the influence of drying after harvest, genetic potential, environmental conditions, date of harvest, mechanical damage, and storage conditions.

Accelerated ageing is a widely accepted vigor test appropriate for large numbers of crop seeds (Hampton and TeKrony, 1995). Maximum germination after an accelerated ageing test shows that the greatest seed vigor occurs in seeds harvested at 75 DAA. This obviously shows that seeds require some days in order to achieve maximum resistance to high temperature drying - advancement in vigor - after completion of the maximum dry weight accumulation. Some previous studies also indicate that seeds of various crops reached maximum seed quality (germination, quality, potential longevity, and seedling emergence) some days after the occurrence of maximum seed dry mass (Demir and Ellis, 1992). This evidently shows that completion of the deposition of storage reserves during development does not necessarily overlap with the occurrence of the maximum quality and in turn high temperature drying of the seeds.

In conclusion, pepper seeds are resistant to high temperature drying as long as they are harvested at 75 DAA. Drying temperature and harvest time combinations do not affect sucrose or fatty acid contents remarkably. Drying at 45 °C reduces glucose and fructose contents compared to those of 25 and 35 °C and their content are at the lowest level at the final harvest in both years.

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