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Effect of gamma irradiation and temperature on fructans (fructo-oligosaccharides) of stored onion bulbs *Allium cepa* L

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Abstract

The effects of gamma irradiation doses and temperatures on fructo-oligosaccharides of onion bulbs after six months storage were investigated. Bulbs were ionized at doses of 0.15 and 0.30 kGy, and kept at 4, 10 and 20 °C during 24 weeks. The concentrations of glucose, fructose, sucrose and other fructo-oligosaccharides were then determined. Fructans content decreased with degree of polymerization (DP), and glucose, fructose and sucrose constituted major proportions of total carbohydrates, averaging 28%, 24% and 10%, respectively. Trisaccharides averaged 12%, while tetra-saccharides averaged 10% of total carbohydrates. High polymerized fructo-oligosaccharides averaged 13% for DP 5–8 and 5% for DP up to 12 U. After six months, glucose, fructose and sucrose of control and both irradiated bulbs decreased slightly but not significantly, while temperature and irradiation significantly influenced fruco-oligosaccharides of bulbs.

Keywords: Fructans; Temperature; Irradiation; Onion

1. Introduction

Onions with other members of the Alliums family are generally consumed for their flavour, and their nutritive value has only been recently appreciated (Salunkhe & Wu, 1974). During their harvesting, handling, transportation, packaging and storage, onion bulbs are exposed to several treatments and environmental conditions which can affect their quality attributes and physiological characteristics. These effects could be responsible for several reactions and stresses, causing important biochemical changes to the bulb tissues (Benkeblia, 2003).

Bulb dry matter content is an important quality parameter of onion, and several investigations have attempted to relate bulb characteristics and storage life (Rutherford & Whittle, 1984). About 80% of bulb dry matter are non-structural carbohydrates (Darbyshire & Henry, 1981). The predominant members of these non-structural carbohydrates are glucose, fructose, sucrose and low-molecular-weight fructans, while starch and raffinose are absent (Benkeblia, Varoquaux, Shiomi, & Sakai, 2002; Darbyshire & Henry, 1981). The metabolism of sugars is closely linked to the dormancy and sprouting state (Kato, 1966), and the most important biochemical changes occurring during long term storage of bulbs, as of other vegetables, are the quantitative variations in the carbohydrate constituents. Variations of mono and disaccharides levels in onion bulbs during storage were previously reported (Benkeblia et al., 2002; Benkeblia & Varoquaux, 2003; Hurst, Shewfelt, & Schuller, 1985; Rutherford & Whittle, 1982); however, variation of fructo-oligosaccharides and the effect of gamma irradiation and long term storage on these constituents were not investigated.

Ionizing radiation is defined as a process in which food products are exposed to a controlled amount of radiant energy. Irradiation increases shelf life of fruits

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and vegetables, and reduces spoilage, and several investigations have been carried out throughout the world on the use of ionizing radiation to control sprouting in onions (Elias & Cohen, 1983; Matsuyama & Umeda, 1983). Irradiation doses ranging from 0.05 to 0.15 kGy inhibit bulb sprouting, and are more effective when applied during the dormancy period, specifically within 4–6 weeks following harvesting (Salunkhe & Wu, 1974). Ionized bulbs can be stored for several months without heavy spoilage, though ionization and storage can affect changes in the carbohydrate contents of onion tissues. However, despite the existence of numerous data on the commercial quality of irradiated onion bulbs, little information is available about the pattern of changes in the main chemical components, such as non-structural carbohydrates, during irradiation treatments and long-term storage.

The aim of this investigation was to assess the effect of irradiation and long-term storage on dry onion bulbs. Two irradiation doses were used (0.15 and 0.30 kGy) and treated bulbs were stored at three different temperatures (4, 10 and 20 °C) for 24 weeks. The concentrations of glucose, fructose, sucrose and other fructo-oligosaccharides were then determined.

2. Materials and methods

2.1. Onions

Dry onion bulbs *Allium cepa* cv. Jaune d'Espagne (organic product, free of any preharvest chemical treatments), which had been freshly harvested and dried in the field for 2 weeks, were obtained from the local market. They were sorted for uniformity and absence of defects, packed in commercial plastic (PVC) trays and placed at 18 °C prior to treatments. Each tray contains 12 kg onions, and three trays were used for each irradiation dose and temperature.

2.2. Ionizing treatment

The irradiation treatment was applied two weeks after harvesting. Ionizing treatment of the onion bulbs was performed using a ^{60}Co source at doses of 0.15 and 0.30 kGy at 20 °C. The exposure times were 30 and 55 min, respectively, and a Fricke dosimeter was used for process control.

2.3. Storage conditions

Immediately after ionizing treatment, onions were stored at three sets of temperatures and relative humidity: 4 °C and 85% RH, 10 °C and 80% RH and, ambient conditions of 20 °C and 65% RH.

2.4. Saccharides extraction and analysis

Glucose, fructose and sucrose contents were determined by HPLC. Samples of 5 g of freeze-dried tissues were homogenized in 50 ml of water, using of Sorvall blender (Omni-mixer 17220, Newton, USA). The homogenate was then heated for 30 min in a boiling water bath (Haake Inst., Berlin, Germany). After cooling, the homogenate was centrifuged for 15 min at 25,000*g* (Heraeus Sepatech GmbH, Osterode/Harz, Germany) and the supernatant was filtered on a 0.25-μm filter (Millipore S.A, Molsheim, France).

The sugars were separated by HPLC, using a Varian 5000 model (Vista, 5000 series, Les Ulis, France) fitted with a Polyspher CH-CA column (300×7.8 mm. Merck, Darmstadt, Germany) set at 80 °C with an appropriate guard column (Merck) and a differential refractometer detector (Knauer GmbH, Hegaver, Berlin, Germany). Mobile phase was DDI water at a flow rate of 0.5 ml min^{-1}.

2.5. Fructo-oligosaccharides extraction

Fructo-oligosaccharides were extracted by the method of Shiomi (1992). Tissues (10 g) were homogenized in 80 ml of aqueous ethanol (70 %) using a small amount of calcium carbonate. The homogenate was boiled under reflux in a water bath during 10 min. Then, homogenate was filtered and the residue extracted three times with aqueous ethanol and one time with water under the same conditions. The filtrates were combined and made up to 500 ml with distilled water. An aliquot of the filtrate (10 ml) was concentrated in vacuo, at 35 °C, to dryness using a Büchi rotavapor. The concentrated sugars were collected in 1 ml of water and passed through a 0.45-μm filter and analyzed by high performance anion exchange chromatography (HPAEC, Dionex, Sunnyvale, CA, USA). All processes were run in triplicate.

2.6. Fructo-oligosaccharides analysis

The saccharides were separated on an HPLC-carbohydrate column PA1, Carbo Pack with a Dionex Bio LC series HPLC (Sunnyvale, CA, USA) and pulsed amperometric detector (PAD). The gradient was established by mixing eluent A (150 mM NaOH) with eluent B (500 mM acetate-Na in 150 mM NaOH) in two ways. System I: 0–1 min, 25 mM; 1–2 min, 25–50 mM; 2–20 min, 50–200 mM, 20–22 min, 500 mM; 22–30, 25 mM. System II: 0–1 min, 5 mM; 1–2 min, 25–50 mM; 2–14 min, 50–500 mM, 14–22 min, 500 mM; 22–30, 25 mM. The flow rate through the column was 1.0 ml min^{-1}. The applied PAD potentials for E1 (500 ms), E2 (100 ms) and E3 (50 ms) were 0.01, 0.06 and −0.6 V, respectively, and the output range was 1 μC.

3. Results

As shown in Fig. 1, glucose, fructose and sucrose constitute a major proportion of non-structural carbohydrates, while tri- and tetra-saccharides contents are lower. It was observed that concentration of high polymerized fructans decreased with their degree of polymerization. The distribution of the carbohydrate constituents is shown in Table 1. It was noted that glucose, fructose and sucrose constitute of a major part of the dry matter and non-structural carbohydrates, averaging 28%, 24% and 10%, respectively. Tri-saccharides, including 1-kestose and neokestose (iso-kestose), averaged 12%, while tetra-saccharides averaged 10% of total carbohydrates. High-polymerized fructo-oligosaccharides averaged 13%, for DP between 5 and 8, whereas other fructans (DP up to 12) constituted less than 5%.

Table 1
Saccharides composition of fresh onion bulbs cv. Jaune d'Espagne

	Low	High	Average ± SD
	(mg g^{-1} dry weight)		
Glucose	176	215	197.5 ± 17.8
Fructose	156	190	174.2 ± 10.6
Sucrose	66	93	82.3 ± 5.8
DP 3			
1-Kestose	29.7	34.7	31.4 ± 3.5
Neokestose	46.2	52.6	48.1 ± 4.1
DP 4			
Nystose	18.5	24.4	21.6 ± 2.8
4b	26.1	29.1	26.7 ± 3.3
4c	21.6	28.3	24.8 ± 3.7
DP 5–8	85	94	89.4 ± 6.9
DP up to 12	27.8	35.3	31.6 ± 5.3

Fig. 2 illustrates the contents of glucose, fructose and sucrose in control and irradiated bulbs stored for six months at 4, 10 and 20 °C. Glucose content was lower than initial content but relatively the same after six months without any significant difference noted between control and 0.15 kGy-treated bulbs, or, between the two irradiated bulbs. On the other hand, significant difference was noted between control and 0.30 kGy-treated bulbs. However, temperature affected the content of glucose in control bulbs, where its concentration was the lowest at 20 °C after six months. At 4 and 10 °C, glucose content of irradiated bulbs decreased non-significantly after the same period of storage.

After six months and comparatively to the initial content, fructose decreased significantly in control and both irradiated bulbs, at 4, 10 and 20 °C; however, this decrease was not significant between control and irradiated bulbs. Irradiation doses and temperatures do not seem to affect fructose content; on the other hand, temperature significantly affected fructose content in control bulbs after six months storage at 20 °C. Sucrose content decreased significantly but did not show difference between control and both irradiated bulbs after six months storage at 4 and 10 °C. However, at 20 °C, sucrose content of control bulbs was significantly lower than of those kept at 4 and 10 °C. The decrease of sucrose is caused by the high catabolism due to the sprouting of onions, and to the long-term storage, responsible of the exhausting of high polymer carbohydrates which are hydrolyzed, producing sucrose and fructose. This fact could explain the relatively high level of fructose observed after six months storage.

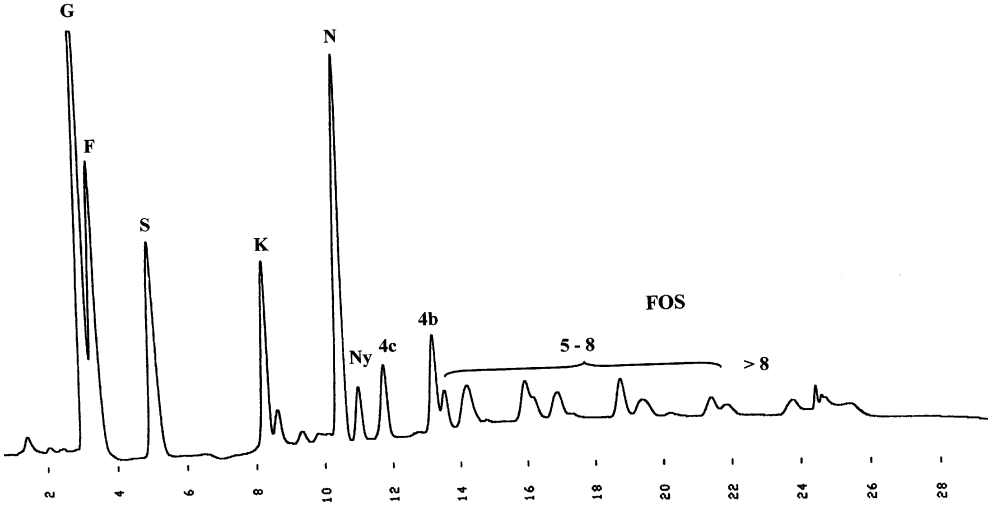

Fig. 1. Chromatogram of saccharides from onion bulb tissue analyzed by HPAEC-PAD. G: glucose, F: fructose, S: sucrose, FOS: fructo-oligosaccharides, K: 1-kestose (1^F-β-D-fructofuranosylsucrose), N: neokestose (6^G-β-D-fructofuranosylsucrose), Ny: nystose (1^F(1-β-D-fructofuranosyl)$_2$sucrose), 4b: 6^G (1-β-D-fructofuranosyl)$_2$ sucrose, 4c: 1^F, 6^G-di-β-D-fructofuranosylsucrose, FOS: fructo-oligosaccharides (1^F(1-β-D-fructofuranosyl)$_m$-6^G (1-β-D-fructofuranosyl)$_n$ sucrose; $2 \leqslant m \leqslant 3$ and $2 \leqslant n \leqslant 4$ for DP 5–8, and $m + n \geqslant 6$ for DP up to 12).

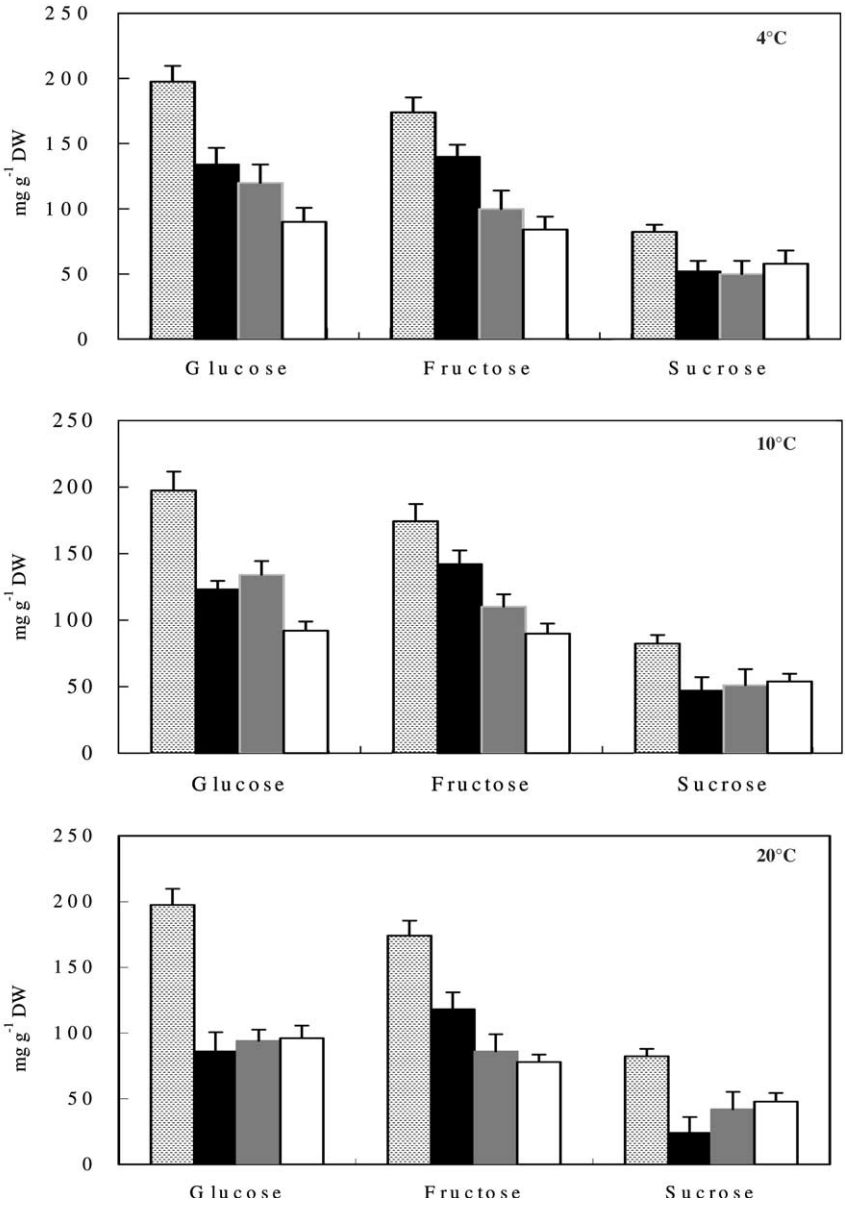

Fig. 2. Effect of irradiation dose and temperature on glucose, fructose and sucrose contents of onion bulbs kept six months at 4, 10 and 20 °C (▨ initial fresh bulbs, ■ control, ▩ 0.15 kGy, □ 0.30 kGy).

As illustrated in Fig. 3, fructans content decreased slightly in both irradiated bulbs; on the other hand, they decreased significantly in controls. At 20 °C, high polymerized fructans – DP 5 to up to 12 – of control bulbs were strongly hydrolyzed, while, at 4 and 10 °C, slight degradation of these polymers and slight accumulation of tri- and tetra-saccharides was noted. This accumulation is the result of low temperature effects which on the one hand slow down the catabolic activity of tissues, and, on the other hand, favour the hydrolysis of fructans. It also seems that tri- and tetra-saccharides play a regulating role between high polymerized and disaccharides, by regulating fructan hydrolase activity and preventing excessive accumulation of fructose as a primary product of hydrolysis and sucrose. In irradiated bulbs, the slight difference observed over time is the result of the low catabolic activity, resulting from the effect of temperature and the damage of gamma rays caused to the cells.

4. Discussion

Variation of mono and disaccharides in onions, particularly irradiated bulbs, was not extensively studied. Salama, Hicks, and Nock (1990) reported a decrease in total sugars and glucose in control onions stored for 5 months at 0, 15 and 30 °C, but fructose increased, particularly at 0 °C. Similar results on fructose were reported by Rutherford and Whittle (1982) at 0 °C.

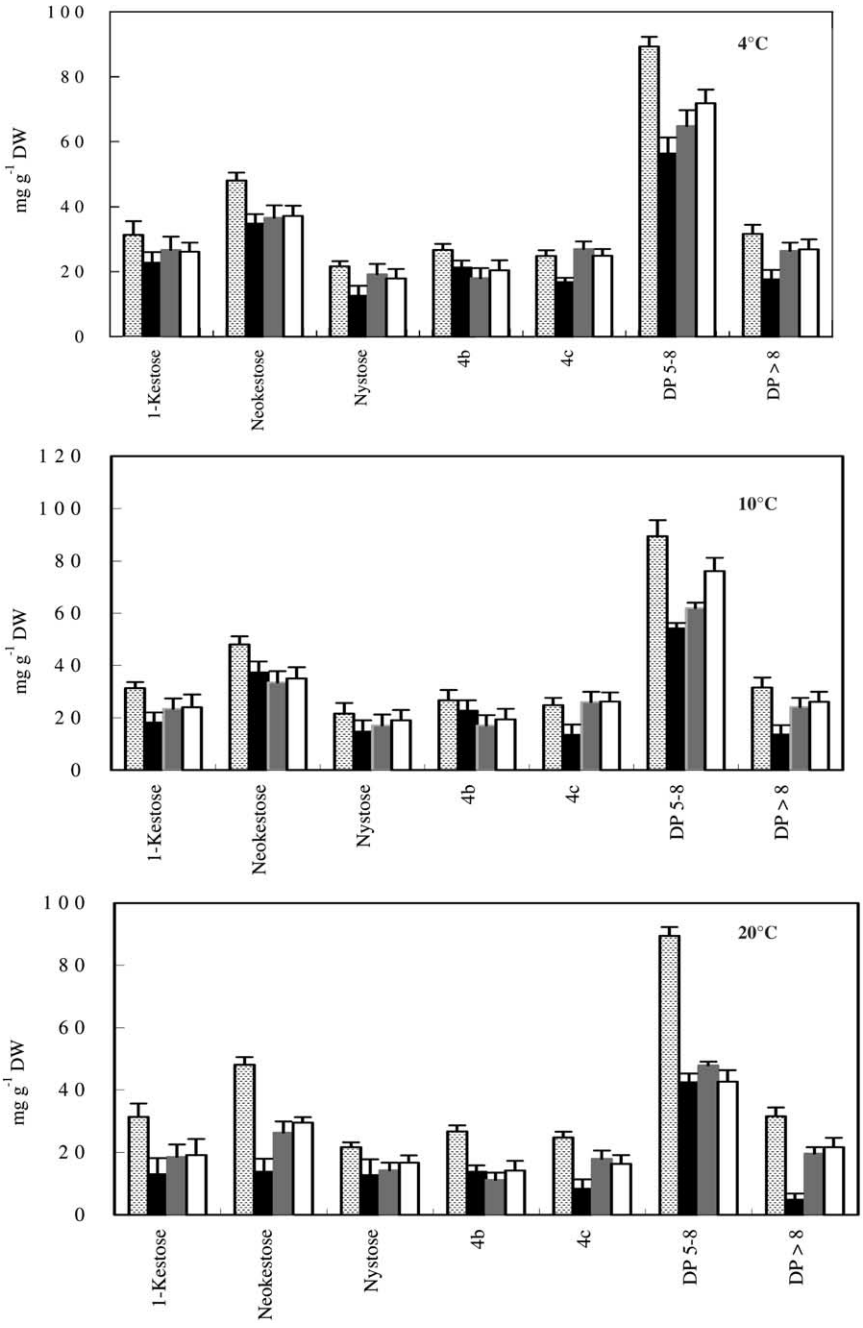

Fig. 3. Effect of irradiation and temperature on fructo-oligosaccharides of onion bulbs kept six months at 4, 10 and 20 °C (▒ initial fresh bulbs, ■ control, ■ 0.15 kGy, □ 0.30 kGy).

Hurst et al. (1985) noted a decrease in total sugars of onion kept during 6 months at 1 and 4 °C, but no variation was noted at 21 °C. Benkeblia et al. (2002) also did not observe any significant difference in total saccharides (glucose, fructose, and sucrose) of control and irradiated bulbs stored for 6 months at 4, 10 and 20 °C. The variation in saccharides, reported by these numerous investigators, suggests that metabolism of sucrose is not clearly understood. This component plays a central role in growth and development of plants, and products of sucrose degradation are important factors, particularly during break of dormancy when internal sprouting could probably be initiated by utilizing the free sugar accumulation (Benkeblia & Selselet-Attou, 1999).

Changes in highly polymerized fructans level are not well documented and their metabolism in onions, and other plants, remains unclear. It was reported that total fructans decreased after 6 months storage independently of temperature, although their degradation is higher at low temperature (Rutherford & Whittle, 1982). Suzuki

and Cutcliffe (1989) reported similar results with a slight increase of some high polymerized fructans (DP 5–8). Benkeblia et al. (2002) noted an increase of fructans, particularly DP 5–8, of onion bulbs after 6 months at 10 and 20 °C. These results apparently contradict those previously reported by some authors. A possible explanation could be on the one hand the difficulty of separating the fructans, and, on the other hand, the presence of multiple isomers of the DP 5 and DP 6 fructo-oligosaccharides, depending on: (1) type of onions, (2) maturity at harvesting, and (3) cultivars. Despite their importance in onion bulbs, and other vegetable crops, the mechanism behind their hydrolysis and enzymes involved, particularly 1-fructoexohydrolase (1-FEH), remains totally unknown. Rutherford and Whittle (1984) found that fructans content at harvest time is a more reliable parameter for predicting storage life of onion bulbs. However, the present knowledge is too limited to explain the catabolism of fructans and the mechanism by which they contribute to storage life. This catabolism does not only depend on the cellular activities during dormancy and sprouting, but also environmental factors that affect the physiological parameters of the bulbs, especially temperature and physical stress, such as ionization (Benkeblia, Varoquaux, Gouble, & Selselet-Attou, 2000).

Finally, it is concluded that temperature and storage time significantly influenced the carbohydrate contents of untreated onions, while carbohydrate contents of ionized bulbs were also influenced by the sprout inhibition. The break of dormancy and sprout growth and elongation may create a greater demand for mobilization and utilization of carbohydrates, particularly glucose and fructose and their direct polymer sucrose. Nevertheless, the metabolism of carbohydrates of onion bulbs, during break of dormancy and sprouting, remains complex, due to the involvement of multiple-degrading enzymes of the different fructo-polymers. Further investigations are needed to determine the nature and the activity of these enzymes, particularly their substrate specificity and their products.

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