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EFFECT OF SOAKING, GERMINATION AND MICROWAVE COOKING ON FLATULENCE-CAUSING OLIGOSACCHARIDES OF CERTAIN LEGUMES

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Abstract: The effect of soaking, germination, ordinary and microwave cooking on oligosaccharides in some commonly legumes namely; faba bean, mung bean, cowpea and kidney bean were investigated. HPLC determination showed that raffinose family oligosaccharides (RFOs); (raffinose, stachyose and verbascose) constituted 47-61% of soluble carbohydrates in the studied legumes. Raw kidney bean contained the highest amount of oligosaccharides compared with other tested legume seeds. Soaking for 12 h in tap water (25ºC±2) led to losses in total RFOs by 21, 24, 23 and 25% in faba bean, mung bean, cowpea and kidney bean, respectively. The highest losses were found in raffinose (26%) and stachyose+verbascose (28%) in mung bean and cowpea, respectively. Germination of seeds indicated that there were significant reductions in the concentration of sucrose and RFOs. As the period of germination was elongated, the concentration of the oligosaccharides declined further. Ordinary and microwave cooking of raw and soaked seeds lowered RFOs content in all investigated legumes. Ordinary cooking of raw and soaked legumes caused losses of total RFOs amounted up to 15-39% and 17-42%, respectively. The reductions of total RFOs were 33-44% after microwave treatment of its initial content in soaked legume seeds. The combined effect of soaking and microwave cooking of seeds caused further removal of RFOs by 42-60% losses of its initial content in raw samples. It may be concluded that germination and microwave cooking of food legumes caused considerable losses of total oligosaccharides as well as reducing flatus-causing agent.

Keywords: Oligosaccharides, soaking, germination, ordinary and microwave cooking, legumes.

Introduction

Legumes contain 2-3 folds more proteins than cereals, besides being good sources of dietary carbohydrates. Plant proteins are now identified as biologically active and functionally versatile dietary components and are cheaper substitutes than animal proteins (FAO, 1990). Legumes are one of the richest and least expensive sources of protein in the human diet in many parts of Egypt. Legumes are high in fiber content that may have hypoglycemic and hypocholesterolemic effects and may reduce risks of colon cancer (Anderson *et al*., 1990). Supplementing cereal-based diets with legumes improves overall nutritional status and is one of the best solutions to protein calorie malnutrition in the developing countries. Legumes also have the potential to lower cholesterol and serum glucose and quicken adjustment to high altitude induced stress (Mazur *et al*., 1998). However, the presence of certain antinutritional factors, such as flatulence causing raffinose family oligosaccharides (RFOs), limits their biological value and acceptance as a regular food item (Reddy *et al*., 1984). Raffinose, stachyose and verbascose (α-galactosyl derivates of sucrose) are associated with desiccation tolerance and storability of seeds (Obendorf, 1997). They produce flatulence in man and animals due to the absence of the enzyme α -galactosidase which is needed for hydrolysis of the α-1,6 galactosidic linkage of these oligosaccharides in the lower intestine. These sugars then undergo anaerobic fermentation by bacteria producing carbon dioxide, hydrogen and small amounts of methane gas that cause flatulence which is characterized by abdominal rumblings, cramps, diarrhea and nausea (Price *et al*.,1988). Degradation of RFOs during germination by endogenously synthesized α -galactosidase, is also well documented (McCleary and Matheson, 1974). Beneficial effects associated with the consumption of legumes are related to the slow rate of starch digestion and the high content of resistant starch in legumes (Truswel, 1992). A wide range of processing techniques such as germination, dehulling, cooking, roasting, autoclaving, fermentation and extrusion have been used and tested to increase the utilization of legumes. Cooking of legumes by microwave has not been extensively studied but it has been shown to reduce antinutritional factors and have positive effects on protein digestibility in others legumes (El-Beltagy, 1996). A study on selected legumes cooked by microwave is thus needed to ascertain whether this treatment could reduce flatus-producing factors and eventually replace traditional cooking which are not only costly in energy but also cause important losses in soluble solids. Therefore, the present study was carried out to determine the effect of soaking, germination, combined soaking and ordinary or microwave cooking on the total soluble sugars and oligosaccharides of legume seeds and to describe a suitable method for reducing flatus-producing factors in legumes.

Materials and Methods

Materials:

Faba bean (*Vicia faba L.*) and Mung bean (*Vigna radiata*) were obtained from Agronomy Department, Faculty of Agriculture, Assiut University. Cowpea (*Vigna sinensis*) and kidney bean (*Paseolus vulgaris*) were obtained from local markets in Sohag city. All legume samples were collected during the 2006 season.

Processing and cooking methods

Soaking: In a glass baker, 500g of clean seeds were soaked in distilled water (1:5, w/v) for 12 h at ambient temperature (25±2ºC). After soaking, the unimbibed water was discarded. Half amount of the soaked seeds was used to germination process and the rest was washed twice with distilled water and then dried in an oven at 70º C for 16 h. The dried seeds were cooled in a desiccator, milled to pass trough a 60-mesh sieve. Legume flours were stored in an air-tight container at -10 ºC until analyzed.

Germination: The presoaked legume seeds were surface sterilized for 30 min in a 1% sodium hypochlorite solution and allowed to sprout on sterile germinating trays lined with filter paper which was kept moist by layers of damp cotton wool. Germination was carried out at 25±2°C in the dark for 4 days. The germinated seeds were dried and milled into flour, as above-mentioned for soaked samples.

Ordinary cooking: 100g of raw or soaked seeds were added to distilled water (1:5 w/v) and cooked at 100ºC in a glass beaker on an electric hot plate for 3 h until cooked soft as felt between fingers. The cooked seeds were allowed to cool at room temperature and drained and then dried at 70 ºC in an electric oven (Gallenkamp, UK) for 16 h. The dried seeds were cooled and milled to pass trough a 60-mesh sieve. Bean flours were stored in air-tight containers at -10 ºC until analyzed.

Microwave cooking: Soaked and unsoaked seeds were cooked (seeds to water ratio was 1:4, w/v) in the microwave oven (Goldstar, Model ER-50540, 2450 MHz, Egypt) capable of generating 800 W power at the frequency of 2450 MHz for 10-15 min until cooked soft as felt between fingers. The temperature of the samples after cooking in the microwave was 107±2 ºC. The samples were allowed to cool at room temperature and drained, then dried and stored as described before.

Analytical methods

Moisture was determined as described in AOAC (1990) by an electric oven (Gallenkamp, UK) at 105ºC.

Extraction and Determination of Soluble sugars was carried out according to the method described by Skulinova et al. (2002), approximately 5 g of ground dry sample was homogenized in 20 ml of ethanol: water (80:20, v/v) and refluxed (boiled) for 60 min; 50 ml acetonitrile was added to the extract that was cooled down and supplied with distilled water to the volume 100 ml, filtered through a membrane filter 0.45 µm pore size and analysed for total soluble sugars and oligosaccharides. The total soluble sugars were estimated spectrophotometrically by the phenol-sulfuric acid method (Dubois *et al*., 1956) using glucose as the standard.

HPLC Determination of oligosaccharides. HPLC method was used to determine sucrose, raffinose, stachyose and verbascose according to Black and Bagley (1978). HPLC-HEWLETT-PACKARD Liquid chromatography series 1050, with HEWLETT-PACKARD R.I. detector HP 1047A and Bio-Rad aminx HPX-87C; column 300 mm x 7.8 mm and Alltech OA-1000 column 300x6.5 mm was used. Operating conditions: mobile phase deionized water and auto-degassed in degasser series: 1050; flow rate 0.6ml/min; temperature (70°C); and injection volume 5 µl.

Statistical analysis. All determinations were carried out in triplicate and represented as mean ± standard deviation. Microsoft Excel was used for statistical analysis. Student's *t*-test was used to distinguish significant differences (at $P < 0.05$) among the mean values.

Results and Discussions

Total sugars and oligosaccharides levels in raw legumes.

Total soluble sugars and oligosaccharides content of the raw legume seeds are given in Table 1. Total sugars which could be extracted from raw legume seeds ranged from 4.10g/100g for cow pea to 5.36 g/100g for kidney bean. HPLC determination showed the presence of sucrose and raffinose family oligosaccharides (RFOs) in all tested legumes. RFOs constituted 47-61% of soluble carbohydrates in these legumes. Faba bean and cowpea showed lowest RFOs content among the studied legume seeds. Stachyose + verbascose were the main oligosaccharides in the studied legumes, mung bean seeds had the highest amount (1.67 g/100g) than other legume seeds.

Table(1): Total sugars and oligosaccharides in some raw legumes (g/100g, dry weight basis)*.

Samples	Total sugars	Sucrose	Raffinose	Stachyose + Verbascose	Total α-Galactosides
Faba bean	4.33±0.02	1.28±0.03	0.81±0.04	1.43±0.01	2.24±0.43
Mung bean	4.16±0.05	1.34±0.04	0.87±0.05	1.67±0.13	2.54±0.56
Cowpea	4.10±0.14	1.44±0.10	0.88±0.08	1.51±0.34	2.40±0.44
Kidney bean	5.36±0.21	1.81±0.05	0.93±0.05	1.60±0.13	2.53±0.47

*Means value of three replicates ± SD.

These results agree with those obtained by others (Fleming, 1981; Abdel-Gawad, 1993 and Oboh *et al.*, 2000). Raffinose content of tested raw legume seeds ranged from 0.81-0.93%.

Effect of soaking on oligosaccharides content in legumes.

Total sugars and oligosaccharides contents remaining in the legume seeds after soaking in tap water are shown in Table 2. Soaking of legumes increased the total sugars content by 6-7% except in mung bean where increment of 17% was obtained. This could be a result of the relative hardness of the seed coat which limits the uptake of water and may prevent the leaching of the α-galactosides into the soak water (Oboh *et al.* 2000). The losses in total RFOs were 21, 24, 23 and 25% of its content in raw faba bean, mung bean, cowpea and kidney bean, respectively. The highest loss in raffinose (26%) and stachyose +verbascose (28%) of original content in raw seeds was found in mung bean and cowpea, respectively. Significant reductions in the α-galactoside contents of soaked common beans, cowpeas, lentils and faba beans, were reported by Abdel-Gawad (1993), the extent of the losses was enhanced as the soaking time increased.

Table (2): Effect of soaking on total sugars and oligosaccharides of legumes (g/100g, dry weight basis)*.

Samples	Total sugars (%)	Sucrose (%)	Raffinose (%)	Stachyose + Verbascose (%)	Loss of RFOs** (%)
Faba bean					
Unsoaked	4.33±0.02	1.28±0.03	0.81±0.04	1.43±0.01	-
Soaked	4.65±0.24 (+7)	0.94±0.05 (-26)	0.64±0.03 (-20)	1.12±0.06 (-21)	21
Mung bean					
Unsoaked	4.16±0.05	1.34±0.04	0.87±0.05	1.67±0.13	-
Soaked	4.87±0.09 (+17)	1.01±0.04 (-24)	0.64±0.02 (-26)	1.30±0.16 (-22)	24
Cowpea					
Unsoaked	4.10±0.14	1.44±0.10	0.88±0.08	1.51±0.34	-
Soaked	4.37±0.16 (+6)	1.25±0.15 (-13)	0.73±0.08 (-17)	1.08±0.18 (-28)	23
Kidney bean					
Unsoaked	5.36±0.21	1.81±0.05	0.93±0.05	1.60±0.13	-
Soaked	5.74±0.20 (+7)	1.44±0.11 (-20)	072±0.05 (-22)	1.15±0.10 (-27)	25

*Means value of three replicates ± SD. **Raffinose family oligosaccharides.

Effect of germination on total sugars and oligosaccharides contents in legumes.

Figure 1 shows the changes in total sugars and individual raffinose family oligosaccharides by germination. Germination of 12 h- soaked legume seeds reduced sucrose and RFOs in all tested samples. As the period of germination was elongated, the concentration of the oligosaccharides declined further. Sucrose contents of legume seeds losses of 10-18% during 24- h germination and reached up to 23-35% of original contents in raw seeds during 72- h germination. RFOs exhibited a similar trend during the germination of faba bean (Fig.1 A), mung bean (Fig.1 B), cowpea (Fig.1 C) and kidney bean (Fig.1 D). The changes in total oligosaccharides content, due to germination, indicated that there were significant reductions in all germinated seeds. The loss in raffinose concentration increased from 5-30 to 26-80% after 24 and 72 h of seeds germination, respectively. Moreover, stachyose + verbascose concentrations

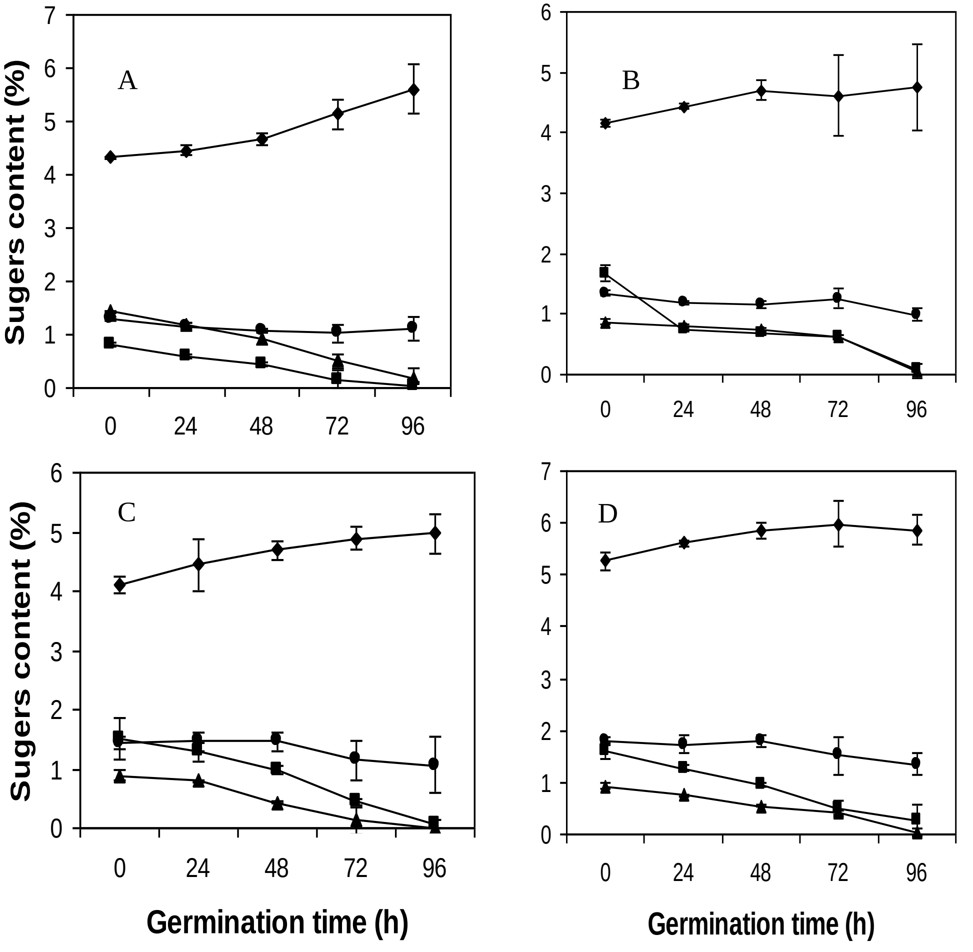

Fig.(1): Changes of total sugars (♦), sucrose (●), raffinose (▲), and stachyose+verbascose (■) during germination of legume seeds. Faba bean (A), mung bean (B), cowpea (C) and kidney bean (D).

were reduced from 21-59 to 61-72% after germination for 24 and 96 h of the studied legume seeds, respectively. In general, the greatest losses of total oligosaccharides were obtained at 96 h of germination. Similar findings have bean reported for related legumes such as black gram and mung beans (Kataria *et al*. 1990). Therefore, germination is one of the most efficient biological treatments for removing oligosaccharides. After germination for 48 h at 20 °C, 40-60% of pea oligosaccharides disappeared (Dostalova *et al*. 2001). Oligosaccharides of the raffinose family are degraded by α-galactosidase which selectively cleaves galactose from raffinose, stachyose and verbascose leaving behind surose. Different legume seeds have variable levels of α-galactosidase activity (Price *et al*., 1988). The fact that sucrose decreased during germination of some legumes indicated that sucrose, in addition to α-galactosides, can be degraded to release energy for the germinating embryo (Oboh *et al*., 2000).

Combined effect of soaking and ordinary cooking on oligosaccharides content in legumes.

Changes in oligosaccharides content after cooking of raw and soaked legume seeds are presented in Table 3. Total soluble sugars content reduced after ordinary cooking of raw seeds of faba bean, mung bean, cowpea and kidney bean by 12-38% of its initial contents, while cooking of soaked seeds increased the losses up to 27-45%. Ordinary cooking of raw legume seeds decreased the sucrose content by 24-65% of its initial contents, whereas, cooking of soaked seeds increased the loses of sucrose content to 43-76%. Ordinary cooking of raw and soaked legume seeds lowered raffinose and stachyose + verbascose contents (Table 3). Ordinary cooking of raw and soaked legumes caused losses of total RFOs amounted up to 15-39% and 17-42%, respectively compared with the corresponding raw samples. Onigbinde and Akinyele (1983) reported a mean decrease of 46 and 50% in the stachyose and raffinose content of 20 cowpea varieties after 45 min cooking. The current findings are in agreement with those reported for cowpeas (Somiari and Balogh, 1993). The combined effects of soaking and cooking led to increased sugar losses in most of the studied legumes, which is in agreement with data reported by Uzogara *et al*. (1996). Total galactosides content of soaked - cooked seeds was significantly lower than raw-cooked seeds in all tested legumes.

Combined effect of soaking and microwave cooking on oligo-saccharides content in legumes.

The results in Table 4 indicated that sucrose content reduced after microwave treatment of raw legume seeds by 28-67% of its initial contents, moreover, microwave cooking of soaked seeds increased the losses of sucrose content to 47-79%. Microwave cooking of raw and soaked seeds lowered also RFOs in the tested legumes. The reductions of raffinose and stachyose+verbascose content by this treatment were 24-43% and 39-49%, respectively. These losses may have been due to heat induced hydrolysis of the oligosaccharides to simple disaccharides and monosaccharides (Onigbinde and Akinyele, 1983). The current findings

Table(3): Combined effect of soaking and ordinary cooking on oligo-saccharides content in legume seeds (g/100g dry weight basis)*

Samples	Treatment	Total sugars (%)	Sucrose (%)	Raffinose (%)	Stachyose+ Verbascose (%)	Loss of RFOs** (%)
Faba bean	Raw	4.33±0.02	1.28±0.03	0.81±0.04	1.43±0.01	-
	Raw-cooked	3.23±0.01	0.79±0.01	0.49±0.01	0.87±0.01	39
	Soaked cooked	3.11±0.01	0.41±0.03	0.41±0.60	0.59±0.01	53
Mung bean	Raw	4.16±0.05	1.34±0.04	0.87±0.05	1.67±0.13	-
	Raw-cooked	3.66±0.07	1.01±0.09	0.66±0.04	1.21±0.05	26
	Soaked cooked	2.74±0.06	0.76±0.04	0.58±0.03	0.99±0.5	37
Cowpea	Raw	4.10±0.14	1.44±0.10	0.88±0.08	1.51±0.34	-
	Raw-cooked	3.06±0.03	0.64±0.02	0.74±0.02	1.24±0.05	15
	Soaked cooked	2.99±0.10	0.46±0.05	0.41±0.03	0.95±0.06	45
Kidney bean	Raw	5.36±0.21	1.81±0.05	0.93±0.05	1.60±0.13	-
	Raw-cooked	3.32±0.03	0.63±0.02	0.72±0.06	1.13±0.01	26
	Soaked cooked	2.92±0.06	0.43±0.03	0.54±0.03	0.91±0.01	43

*Means value of three replicates ± SD.**Raffinose family oligosaccharides.

Table (4): Combined effect of soaking and microwave cooking on oligo-saccharides content in legume seeds(g/100g dry weight basis)[1].

Cultivars	Treatment	Total sugars (%)	Sucrose (%)	Raffinose (%)	Stachyose+ Verbascose (%)	Loss of RFOs[2] (%)
Faba bean	Raw	4.33±0.02	1.28±0.03	0.81±0.04	0.43±0.01	-
	Raw-cooked	3.27±0.02	0.72±0.02	0.46±0.03	0.82±0.05	44
	Soaked cooked	2.39±0.01	0.33±0.02	0.34±0.03	0.44±0.04	60
Mung bean	Raw	4.16±0.05	1.34±0.04	0.87±0.05	1.67±0.13	-
	Raw-cooked	3.63±0.02	0.97±0.05	0.63±0.02	1.01±0.08	33
	Soaked cooked	2.50±0.05	0.70±0.02	0.55±0.03	0.87±0.06	42
Cowpea	Raw	4.10±0.14	1.44±0.10	0.88±0.08	1.51±0.34	-
	Raw-cooked	3.08±0.04	0.54±0.10	0.53±0.02	0.83±0.07	42
	Soaked cooked	2.65±0.05	0.30±0.02	0.44±0.04	0.72±0.06	51
Kidney bean	Raw	5.36±0.21	1.81±0.05	0.93±0.05	1.60±0.13	-
	Raw-cooked	3.24±0.05	0.58±0.02	071±0.02	0.81±0.08	37
	Soaked cooked	2.59±0.02	0.41±0.04	0.45±0.04	0.56±0.02	58

[1]Means value of three replicates ± SD. [2]Raffinose family oligosaccharides.

are in agreement with those reported for peas (Skulinová *et al.* 2002) and chickpeas (El-Adawy, 2002). The combined effect of soaking and microwave cooking of seeds caused further removal of RFOs by 42-60 % losses of its initial content in raw samples. These results are in the line with those reported by Kadlec *et al*. (2001) and Khatoon, and Prakash (2004). They reported that the losses in RFOs may be caused by ongoing hydrolysis by thermal decomp-osition and Maillard reaction between sugars and amines.

Generally, it may be concluded that the domestic methods as well as microwave treatment substantially reduced raffinose family oligosaccharides in the studied legume seeds. Besides, germination and microwave cooking of food legumes caused the greatest loss of total oligosaccharides; RFOs and could be recommended as a suitable method for reducing flatus- causing agents in legumes.

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تأثير النقع ، الإنبات والطبخ بالميكروويف على محتوى بعض البقوليات من سكريات الأوليجو المسببة للانتفاخ

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تم فى هذا البحث دراسة تأثير كل من عمليتي النقع والإنبات ثم الطبخ التقليدي أو الطبخ بالميكروويف علي بذور بعض البقوليات الأكثر انتشارا في مصر، وهى الفول البلدى ، فول المانج ، اللوبيا والفاصوليا الجافة. وقد أوضح تقدير السكريات باستخدام جهاز الـ HPLC أن سكريات الاوليجو المعروفة بسكريات عائلة الرافينوز (RFOs) وهي الرافينوز والاستاكيوز + الفرباسكوز كانت تمثل 47 - 61% من محتوى الكربوهيدرات الذائبة فى هذه البقوليات. وقد احتوت بذور الفاصوليا الجافة أعلى كمية من سكريات الاوليجو مقارنة ببذور البقوليات الأخرى. وعند نقع بذور البقوليات تحت الدراسة لمدة 12 ساعة فى الماء العادي (25 °م ± 2) أدى ذلك إلى تقليل المحتوي من سكريات الاوليجو الكلية بمقدار 21 ، 24 ، 23 و25% فى بذور كل من الفول البلدى ، فول المانج ، اللوبيا والفاصوليا الجافة ، على التوالى. وقد لوحظ أعلي فقد في محتوي سكر الرافينوز (26%) والاستاكيوز + الفرباسكوز (28%) في بذور كل من فول المانج واللوبيا علي التوالي. وعند اجراء عملية الانبات للبذور أدى ذلك الى انخفاض معنوي في تركيز كل من السكروز و سكريات عائلة الرافينوز علي حد سواء. وقد لوحظ انه بزيادة فترة الانبات انخفض تركيز هذه السكريات في البذور تبعا لذلك . وقد أدت عملية الطبخ العادي أو الطبخ بالميكروويف لكل من البذور الخام أوالمنقوعة إلى خفض محتوى سكريات عائلة الرافينوز في جميع البقوليات تحت الدراسة. وقد أدي الطبخ العادي لبذور البقوليات الخام والمنقوعة إلي خفض محتواها من سكريات RFOs الكلية بلغت نسبته 15-39 % و17-42% علي التوالي. بينما كان اختزال سكريات RFOs الكلية تتراوح نسبته بين 33-44% من محتوي بذور البقوليات المنقوعة، بعد الطبخ بالميكروويف.

وقد أظهرت النتائج أيضاً أن التأثير المشترك لعمليتي النقع و الطبخ بالميكروويف لبذورهذه البقوليات كان أكثر وضوحاً في خفض محتواها من سكريات RFOs وقد تراوح هذا الفقد بين 42-60% من المحتوي الأصلي في العينات الخام.

ومن خلال هذه الدراسة يمكن استنتاج ان عمليتى الانبات والطبخ بالميكروويف لبذور البقوليات الغذائية تؤدى الى فقد ملموس ومعتبر في محتواها من سكريات الاوليجو الكلية والمسببة للغازات، ومن ثم تقليل الانتفاخات الناتجة عن تناول تلك البذور غير المعاملة.