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EVALUATION OF SOME PHYSICAL,CHEMICAL AND NUTRITIONAL CHARACTERISTICS OF SOME EGYPTIAN CITRUS SEEDS .

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ABSTRACT

Seeds of mandarin, grapefruit,, sour orange, sweet orange and lime were analyzed and characterized in terms of physical properties, chemical composition, fatty acids and amino acids contents. Trails were made for minimizing the bitterness of the dehulled seeds by soaking in citric acid and boiling in water before using in preparing of cake at 0, 5, 10 and 15% levels. The influence of using such levels of citrus seeds on some cake properties were also evaluated. Among the citrus fruits, the smaller size one had the highest seed content and the lowest seed index. The seeds colour ranged from light yellow to light creamy. They were found to be a good source of crude fat (52.8-57.2%), crude protein (14.78-18.55%), crude fibers (14.25-16.07%), carbohydrates (10.55-12.25%) and total ash (2.10-2.34%). The ratio of unsaturated to saturated fatty acids ranged from 2.08 to 2.45 in citrus seed oils. Except methionine, the other essential amino acids were high in citrus seed protein. Soaking in 3% citric acid caused a reduction in bitter taste feeling of the sour orange . The addition levels of the dehulled sour orange before and after debittering increased yellowness of the prepared cakes. Panelists preferred cakes containing 5% debittered citrus seed flour due to its similarity with the control. Slight increase in both fat and protein of cakes was observed due to addition of 5% debittered sour orange flour.
Keywords; Citrus seeds, Debittering, Supplemented cake, Chemical composition.

INTRODUCTION

At the present time, attention has been baied on the utilization of wastes and by-products of food processing to increase the available food resources and to supplement several products which used as human foods. Furthermore, it can contribute to solve problem of waste disposal and thus minimize the environment pollution (Abdel-Nabey and Attia, 1998).

Among the Egyptian processed fruits, citrus represented the first fruits raw materials (Moharram *et al*., 1997). The seedy varieties of citrus contained 3.5% seed (Moharram, 1980). However, citrus seeds which rich in both oil and proteins, are usually thrown away as a waste of the juice extraction industries (Ali, 1999).

According to Von Woedtke *et al.* (1999), grape fruit seeds extract showed a high growth inhibiting activity against the bacteria, yeasts and molds. Park *et al.* (1998) observed the antimicrobial effects of grape fruit seed extract on spoilage microorganisms isolated from spoiled soybean sprouts.

Bocco *et al.* (1998) suggested the use of citrus seeds as a natural antioxidant due to the occurrence of such substances in these seeds. The antioxidative activity of the grape fruit, orange and lemon seeds extracts was assessed by Pereira and Mancini (1994). Grape fruit seeds extract contained tocopherols, citric acid and ascorbic acid. These components acted as a prooxidant in vegetable oils subjected to oxidation at 97.8 and 75°C (Armando *et al*., 1998).

Also, grapefruit seeds extract considers a superior source of a complex organic plant compounds named bioflavonoids, which function as a co-factor to the body's immune response against inflammation, allergy and infection (Miyake *et al*., 1993 and Sakmoto *et al*., 1996).

The citrus seed limonoids possess an important biological activities as anticancer. They had anticancer activity in laboratory animals, ability to inhibit cell proliferation cancer and human breast cancer cells (Tian and Schwartz, 2003).

Sreenath *et al.* (1995) used citrus fruit seed extract as a natural colouring and as a clouding agent during soft drinks preparation. Ali (1999) utilize the defatted, debittered mandarin seed flour as a protein supplier to replace 10% of wheat flour of noodle. The obtained product had an excellent sensory and physical properties.

Studying the physical properties, chemical composition and nutritive value of citrus seeds helps to utilize these seeds or their debittered flour in some food applications. In the present work, trails were made to debitter and evaluate the technological, chemical and nutritional value of the seeds of the main five varieties of Egyptian citrus fruits. Utilization of the debittered seeds in preparing cake was also investigated.

MATERIALS AND METHODS

Materials:

Thirty kilogram samples of the following fresh citrus fruits namely mandarin (*Citrus reticulata*), grape fruit (*Citrus paradisi*), sour orange (*Citrus aurantium*), sweet orange (*Citrus sinensis*) and lime (*Citrus aurantifolia*) were brought from the local Alexandria markets, Egypt in January, 2002. The fruits were cut into halves to extract juice and remove seeds. The seeds were left at room temperature to dry then dehulled manually to get the kernels. The obtained kernels were ground to pass through 60 mesh-sieve and stored in glass kilner jars at -18°C until used. Wheat flour (72% extraction rate), sucrose, egg, shortening, dried milk and baking powder were brought from super markets at Alexandria, Egypt.

Methods:

I-Technological methods:

a-Debittering of dehulled citrus seed flour:

1-Boiling in water: Dehulled seeds were mixed with tap water at 1:5 w/w ratio and boiled for 15 min. After cooling, the dehulled seeds were filtered, then dried at 55°C in an air oven dryer and their bitter taste was examined subjectively (Attia,2000).

2-Soaking in citric acids: 1%, 3% and 5% citric acid solutions were used as a soaking medium for debittering citrus seeds at a 1:5 w/w ratio seed/soaking medium. During soaking, the seeds were subjected for continuos agitation for 30 min at room temperature, filtered, washed by distilled water and dried in an air dryer at 55°C before subjecting to evaluate their bitter taste subjectively (Swisher,1958).

b-Cake preparation: Cake was manufactured as described by El-Din *et al.* (1992) using 250g wheat flour, 275g sucrose, 150g whole liquid eggs, 200g shortening, 200g dried milk, 10g backing powder. The dehulled citrus seeds were used to replace 0, 5, 10 and 15% of the wheat flour weight before and after debittering.

II-Physical methods:

The percentage of seeds in fruits, seed index (g/100 seeds), hull and kernel percentages were determined according to the methods described by Ali (1999). The colour of seed flour and cake was measured by Lovibond Schofield Tintometer, Sales. Ltd., Salisburg, England as mentioned by Mackinnery and Little (1962). Texture of 10 replicates of cake samples was measured using the Universal Pentrometer, Precission, Scientific Company, Chicago, USA, as mentioned by Hartman (1976). The average of 10 readings was reported to express the texture.

III-Analytical methods:

Moisture content, ether extract, crude protein (N X 6.25), crude fiber and ash were carried out according to the AOAC (1990).

Fatty acid methyl esters of citrus seed oil were performed according to the procedure of Radwan (1978), using 1% sulphuric acid in absolute methanol. The obtained fatty acid methyl esters were separated by Shimadzu Gas Chromatograph (GC4CM,PFE)with a flame ionization detector.Standard fatty acid methyl esters were used for identification. The area under each peak was measured by the triangulation method and percentage of each fatty acid was expressed with regard to the total area.

Amino acids composition of citrus seed flours were determined according to the method of Duranti and Cerletti (1979) using the Beckman amino acid analyzer (Model 119CL). Tryptophan was determined colorimetrically by the method of Dalby and Tsai (1975).

IV-Sensory evaluation:

The prepared cakes were cut after cooling, into slices to evaluate their organoleptic properties. The crumb and crust colour, taste, texture and overall acceptability of the cakes were subjectively assessed by ten panelists from Food Science and Technology Department, Faculty of Agriculture, Alexandria University, using a numerical scale by a rating of 1-10 (optimum=10, very poor=1) according to Ahmed (2003).

RESULTS AND DISCUSSION

1-Some properties of citrus seeds:

Data in Table (1) showed that there is a relationship between citrus fruits size and their content of seeds and also some properties of their seeds. Generally, smaller citrus fruits size, lime and mandarin, had the highest seed content and the lowest size (seed index). Lime seeds had the highest hull and the lower kernel percent among the studied citrus seeds.

The Lovibond colour of the citrus fruit seeds composed of yellow colour as a dominant, red colour as a complementary one, and very low value of the blue colour. According to the proportion of these three colour fractions, the colour of citrus seeds ranged from nearly light yellow to light creamy.

Generally, the results stated in Table (1) did not far than those mentioned by Fong *et al.* (1989) and Gafer (1995) for orange seeds, Abdel-Rahman (1980) and Ali (1999) for mandarin seeds. Tsuyki *et al.* (1984) showed that seed index of Japanese citrus seeds varied from 16-18 g/100 seeds according to species.

2-Proximate composition of citrus seeds:-

As seen from Table (2) the main component of the citrus seed kernels was crude fat (52.8-57.2%), followed by crude protein (14.78-18.55%), crude fiber (14.25-16.07%), carbohydrates (10.55-12.25%) and total ash (2.10-2.34%), respectively. Noticeable differences were noticed in proximate composition of citrus seed kernels due to species. Generally seed kernels of sweet and sour oranges contained the highest fat content and the lowest protein value, while mandarin seeds had the highest level of crude fiber, lowest value of ether extract and total ash. On other hand, except protein which is relatively higher in lime seed kernels, both grape fruit and lime seed kernels considered a moderate source of crude fat and crude fiber. Moussa (1990) noticed variations in proximate composition among citrus seed varieties. Gafer (1995) found that orange seeds were rich in crude lipids and crude fiber. Akpata and Akubor (1999) showed that dehulling of sweet orange seeds increased from their content of protein, fat and lowered their values of crude fiber and ash.

3-Fatty acids composition of citrus seed oils:

As illustrated from Table (3), unsaturated fatty acids in citrus seed oil represented more than 65% of their total fatty acids. Therefore, the ratio of unsaturated to saturated fatty acids ranged from 2.08 to 2.45. Among the unsaturated fatty acids, linoleic acid ($C_{18:2}$) was the dominant one, followed by oleic ($C_{18:1}$), linolenic ($C_{18:3}$) and palmitoleic ($C_{16:1}$) fatty acids, respectively. Palmitic acid ($C_{16:0}$) was the major saturated fatty acids in citrus seed oils followed by stearic ($C_{18:0}$) and arachidic ($C_{20:0}$) acids, respectively.

Among the fatty acids composition of citrus seeds, oil of sweet orange seed was the richest in C16:0 and $C_{18:2}$, while lime seed oil contained the highest level of $C_{18:3}$ and lowest value of $C_{18:1}$.

Table (1) General properties of citrus seeds.

Properties / Citrus seeds	Seeds in fruit (%)	Seed index (g/100seeds)	Seed composition (%)		Lovibond colour		
			Hulls	Kernels	Yellow	Red	Blue
Mandarin	9.450	9.782	22.143	77.857	42.7	0.9	0.1
Grapefruit	4.947	16.573	27.742	72.258	40.5	1.7	0.1
Sour orange	3.627	20.194	24.606	75.394	40.0	1.9	0.0
Sweet orange	6.500	23.079	23.168	74.832	23.9	1.6	0.1
Lime	14.758	7.263	39.484	60.516	20.6	1.5	0.0

Table (2) Proximate composition of dehulled citrus seeds.

Citrus seed / Component (%)	Mandarin	Grape fruit	Sour orange	Sweet orange	Lime
Moisture	14.05±0.15	11.21±0.12	12.0±0.22	11.11±0.54	10.84±0.13
Crude protein [a] Total (N_2 X 6.25)	16.78±0.87	16.68±0.65	15.09±0.13	14.78±0.30	18.55±0.80
Ether extract [a]	52.80±1.65	53.77±1.33	55.58±0.38	57.20±0.92	53.46±1.70
Crude fiber [a]	16.07±0.11	15.45±0.13	15.62±0.14	14.25±0.19	15.09±0.14
Carbohydrate [a,b]	12.25	11.33	11.45	11.53	10.55
Total ash [a]	2.10±0.09	2.12±0.11	2.26±0.12	2.23±0.08	2.34±0.1

a: On dry weight basis. **b: By difference.**

Table (3) Fatty acids composition of citrus seed oil.

Fatty acid (%)	Mandarin	Grapefruit	Sour orange	Sweet orange	Lime
A-Saturated :-					
Palmitic ($C_{16:0}$)	24.60	25.23	23.79	27.02	24.29
Stearic ($C_{18:0}$)	6.51	7.04	5.56	5.00	4.37
Arachidic ($C_{20:0}$)	0.29	0.24	0.12	0.24	0.30
Total saturated **(S)**	31.40	32.51	29.47	32.26	28.96
B-Unsaturated :-					
Palmitoleic ($C_{16:1}$)	0.36	1.47	1.39	1.04	0.49
Oleic ($C_{18:1}$)	26.63	25.81	27.33	21.45	20.98
Linoleic ($C_{18:2}$)	37.99	37.75	33.90	39.89	36.13
Linolenic ($C_{18:3}$)	3.62	2.46	7.91	5.36	13.44
Total unsaturated **(U)**	68.60	67.49	70.53	67.74	71.04
U/S ratio:-	2.18	2.08	2.39	2.10	2.45

Generally, the above data agree with those mentioned by Abdel-Rahman (1980), Lazos and Servos (1988), Romero *et al.* (1988), Gafer (1995) for different types of orange seeds and Ali (1999) for oil of mandarin seeds.

4-Amino acids pattern of citrus seed protein:

As shown from Table (4) among the determined essential amino acids in citrus seed protein, leucine + isoleucine present in highest concentration followed by valine, phenyl alanine, lysine, threonine, methionine and tryptophan. Meanwhile, the main three nonessential amino acids in the protein of citrus seeds were glutamic acid, aspartic acid and histidine, respectively.

Among the citrus seed proteins, the highest value of the essential amino acids was found in grapefruit, lime, sour orange, sweet orange and mandarin, respectively. Comparing with FAO provisional amino acid pattern (1973), citrus seed proteins were high in the essential amino acids except methionine. Due to their high level of lysine, the flour of these seeds can be used for supplementation of cereal products. Generally, the data of amino acids in this study were very closed to those obtained by other investigators such as Moussa (1990), Gafer (1995) and Ali (1999). The latter author showed that the limited amino acids in mandarin seed protein were sulfur containing amino acids and tryptophan.

Debittering of citrus seeds:

Ali (1999) mentioned that lemoniods are responsible of the development of the bitterness in citrus seeds. These compounds are a group of complex triterpenoids in an aglycone and glucoside forms (Tian and Schwartz, 2003). Maier *et al.* (1969) isolated the limonin-D-ring lactone hydrtolase enzyme from orange seeds. This enzyme catalyzes the hydrolysis of the limonin mono lacton, non bitter component, to bitter limonin. Treating the surface of the detached citrus fruit with an aqueous solution of 2-Chloroethyl phosphoric acid for 5 days caused 20-40% reduction in their contents of limonin.

In this study and as shown from Table (5), soaking of orange seed flour in 3 or 5% citric acid for 30 min. lowered the bitter taste potency of the seeds. This may be due to, a)the leaching out of the bitter substances in soaking medium, b)changes in structure or hydrolase of the bitter substances in orange seed flour into non bitter components due to the acidic nature of citric acid, pH 3.8-4, which may be enhanced from the hydrolysis of the lactone ring of limonin compound and/or c) activation and acceleration of the action of limonin-D-ring lacton hydrolase and sequentially the formation of limonin, bitter substance, which either easily leached out and/or hydrolysed in soaking medium. Swisher (1958) stated that adjusting the pH of citrus juice either to pH 6 and/or to 3.8-4 caused the disappearance of the juice bitterness. On the other hand, boiling of seed in water did not affect the bitterness of orange seeds (Table 5). Both the bittering treatments improved from the colour of orange seeds. They reduced from both yellow and red colour fractions of seed colour. The high reduction in yellow colour fraction led to appear the treated seed with light creamy colour. The recent study by Tian and Schwartz (2003) showed the importance biological function of limonoids as anticancer agent in laboratory animals.

Table (4) Amino acid composition of citrus seeds.

Amino acids (g/100g protein)	Mandarin	Grapefruit	Sour orange	Sweet orange	Lime	FAO*
Threonine	5.97	4.71	4.02	4.64	3.97	2.80
Methionine	1.08	1.00	1.42	2.21	0.95	2.20
Isoleucine + Leucine	9.49	11.65	9.94	9.71	11.16	9.20
Phenyl alanine	4.24	5.72	5.25	5.03	5.35	2.80
Lysine	4.56	4.26	5.11	3.69	4.85	4.20
Valine	5.87	6.93	6.77	5.44	7.58	4.20
Tryptophan	0.64	0.88	1.13	1.21	0.97	0.60
Serine	5.13	5.55	3.88	4.90	3.00	-
Glycine	4.05	3.80	4.69	5.88	3.83	-
Tyrosine	2.95	3.26	3.01	3.59	3.17	2.8
Histidine	2.25	2.84	2.99	3.26	2.28	-
Arginine	10.78	12.49	12.09	8.81	13.32	-
Aspartic acid	12.71	11.07	9.78	11.23	10.36	-
Glutamic acid	21.45	17.63	19.24	24.05	16.35	-
Proline	3.80	1.73	5.08	1.64	7.27	-
Alanine	3.72	5.14	4.61	3.69	4.07	-

*** FAO provisional pattern (1973).**

Table (5): Changes in bitter taste and lovibond colour of sour orange seeds after debittering by boiling in water and soaking in citric acid.

Treatments	Bitter taste potency	Lovibond colour		
		Yellow	Red	blue
A-Control	++++	40.0	1.9	Zero
B-boiling in water	++++	20.1	1.2	Zero
C-Soaking in;				
1% citric acid	++	20.0	1.5	Zero
3% citric acid	+	20.0	1.4	Zero
5% citric acid	+	20.0	1.4	Zero

5-Properties of cake supplemented with citrus seed flour:

Table (6) and Fig (1) illustrate the colour, penetrometer value and organoleptic properties of cake supplemented with 0, 5, 10 and 15% of dehulled sour orange seed flour before and after debittering with citric acid. Data in Table (6) indicated that increasing the addition levels of sour orange seed flour from 5 to 15% increased from the yellowness and decreased from the penetrometer reading of the prepared cake. Generally, the cake containing debittered seeds had a lighter pale yellow than those having the bitter seeds .

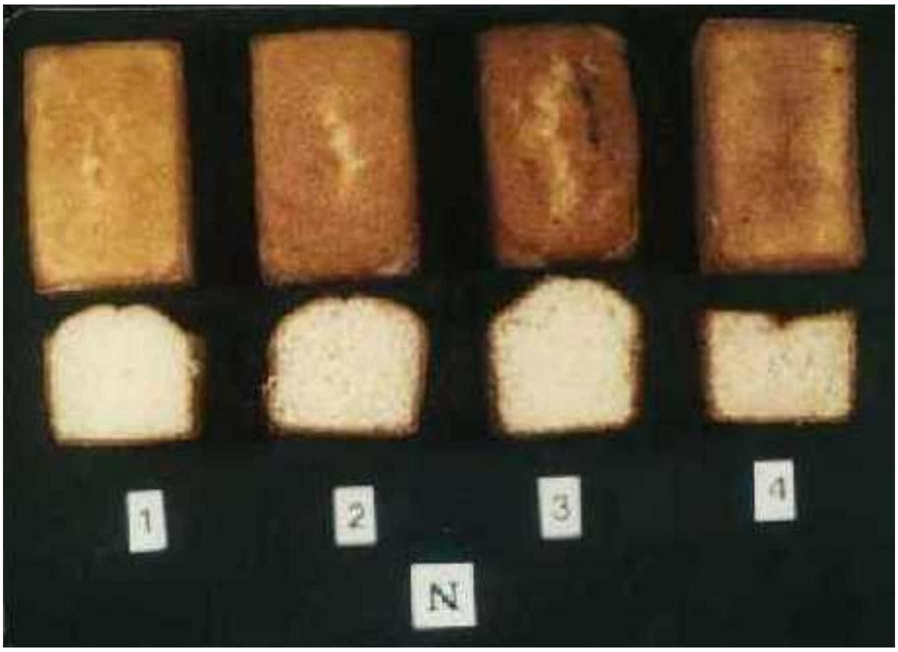

Fig (1) Cake containing different levels of sour orange seed flour.
1- Control 2- 5% 3- 10% 4-15%

Panelists preferred the taste and odour of the cake containing debittered seed flours than those having bitter seeds. The degree of this preference was high in cakes containing 5% debittered seed flour. The sensory properties of this type of cake was very closed to the control, free from sour orange seeds.

Data in Table (6) showed the proximate composition of the supplemented cake with 5% debittered sour orange seeds flour. Generally, slight increase in both fat and protein of cake was observed due to this supplementation. Gafer (1995) suggested addition of citrus seed flour at 1% level to pancakes and beef burgers. At this level of addition, the quality attributes of these products did not affect.

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تقييم بعض الخصائص الفيزيقية و الكيماوية و التغذوية لبعض بذور الموالح المصرية.

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قسم علوم وتكنولوجيا الأغذية- كلية الزراعة - الشاطبى- جامعة الاسكندرية

تم تقييم و دراسة بعض الصفات الطبيعية،التركيب الكيماوى،الأحماض الأمينية والأحماض الدهنية لبذور ثمار اليوسفى، الجريب فروت، النارنج، البرتقال والليمون . كما أجريت محاولات لتقليل مرارة البذور المقشورة بالنقع فى حامض الستريك والغليان فى الماء وإحلالها بنسب مختلفة من دقيق القمح (صفر،٥،١٠،١٥ %) فى صناعة الكيك مع دراسة تأثير نسب الإحلال على بعض خواص الكيك. وقد وجد أن ثمار الموالح الأصغر حجما تحتوى على أعلى نسبة من البذور ويتراوح لون البذور عموما ما بين الأصفر الفاتح للكريمى الفاتح. و لقد أظهرت الدراسة أن تلك البذور تعتبر مصدراً جيداً لكل من الزيت الخام (٥٢,٨ – ٥٧,٢%) والبروتين الخام (١٤,٧٨ –١٨,٥٥%) و كذلك الألياف الخام (١٤,٢٥ –١٦,٠٧%) والكربوهيدرات (١٠,٥٥ –١٢,٢٥%) و الرماد الكلى (٢,١٠ – ٢,٣٤ %) . هذا و قد تراوحت نسبة الأحماض الدهنية الغير مشبعة إلى الأحماض الدهنية المشبعة ما بين ٢,٠٨ إلى ٢,٤٥ فى زيوت بذور الموالح . و فيما عدا الميثيونين فقد لوحظ ارتفاع نسبة الأحماض الأمينية الضرورية فى بروتينات البذور. ولقد أدى نقع بذورالنارنج المقشورة فى ٣% حامض سيتريك إلى اختزال الإحساس بالطعم المر ، كما أسفرت زيادة نسب الإحلال بدقيق بذور الموالح المقشورة سواء المرة أو المزالة المرارة إلى زيادة اللون الأصفر و تغير قوام الكيك المصنع . ولقد فضل المحكمون عينات الكيك المحتوى على نسبة ٥% من دقيق بذور النارنج المقشورة المزالة المرارة حيث أعطت صفات حسية مشابهة لعينة الكنترول . كما أظهرت الدراسة زيادة بسيطة فى محتوى الكيك المصنع من كل من البروتين و الزيت كنتيجة للإحلال ببذور الموالح .

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Table (6) : General properties of cake supplemented with different concentrations of bittered and debittered dehulled sour orange seed flour.

Supplement Concentration (%) / Properties	Control	Bittered seed flour			Debittered seed flour		
		5	**10**	**15**	**5**	**10**	**15**
Lovibond colour:-							
Yellow	10.10	11.10	21.10	21.10	8.20	6.10	5.30
Red	1.20	1.30	1.20	1.25	1.10	1.10	1.10
Blue	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Penetrometer reading (50g/cm)	2.756±0.54	2.924±0.69	2.632±0.64	2.010±0.60	2.607±0.30	2.429±0.43	1.972±0.22
Organoleptic properties:-							
Colour							
Crumb	8.00±0.71	8.40±0.71	8.00±0.77	7.30±1.01	8.40±0.49	8.40±0.49	7.80±0.75
Crust	8.50±0.75	8.60±0.87	8.18±0.83	7.73±0.96	8.60±0.49	8.40±0.49	8.20±0.98
Flavour							
Odour	8.30±0.67	8.10±0.78	7.00±0.78	5.75±0.83	8.60±0.80	8.50±0.76	8.13±1.11
Taste	8.90±0.70	7.13±0.78	5.80±0.87	5.27±0.94	9.20±0.49	7.25±0.43	6.50±1.17
Texture	9.00±0.49	8.40±0.92	7.13±0.60	6.70±0.90	9.10±0.40	8.50±0.69	7.90±1.02
Overall acceptability	8.540	8.106	7.222	6.550	8.780	7.806	7.706
Proximate composition:-							
Moisture content	27.40±0.51	-	-	-	27.65±0.35	-	-
Crude protein [a]	8.76±0.15	-	-	-	9.57±0.17	-	-
Crude fat [a]	22.85±0.53	-	-	-	23.82±0.84	-	-
Ash [a]	1.23±0.02	-	-	-	1.27±0.02	-	-
Total carbohydrate [a, b]	39.76	-	-	-	37.69	-	-

a = On dry weight

b = By difference.