MERIT RESEARCH JOURNALS
www.meritresearchjournals.org

Merit Research Journal of Agricultural Science and Soil Sciences (ISSN: 2350-2274) Vol. 1(3) pp. 042-047, October, 2013
Available online http://www.meritresearchjournals.org/asss/index.htm

Full Length Research Paper

Functional properties and In-vitro digestibility of bitter orange (*Citrus aurantium*) seed flour

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Abstract

Due to the severe shortage of animal protein, especially in developed countries, research has focused on providing new sources of plant proteins for the lack of this gap. On the other hand, industrial citrus processing creates large quantities of waste which have an adverse environmental impact. So, this work was performed to valorized bitter orange seed proteins including its functional properties and amino acid composition as a source of edible plant protein. The results revealed that foam capacity of bitter orange seed flour (BOSF) proteins was better at acidic medium (pH4), while, its stability was better at natural medium (pH7). Also, emulsion, water holding and oil holding capacities of BOSF were 15.5 ml/g, 2.6 ml/g and 2.1 ml/g. Moreover, good gel was formed at 10% (w/v) of heated and defatted BOSF. Crude protein and nitrogen protein content were 17.9% and 12.96%, respectively. Protein solubility, digestibility and amino acids composition of protein were also determined to evaluate the protein quality. The first limiting amino acid was Lysine 48.8% and second limiting amino acid was Isoleucine 77.1% comparing with reference protein. The protein efficiency ratio (PER) was 1.1.

Keywords: bitter orange seed, functional properties, nitrogenous compounds, Amino acids composition

INTRODUCTION

Mediterranean countries produce significant amounts (31.2 million Mt) of orange and other citrus fruits (FAO, 2004). Citrus is an important fruits with annual world production about 53.8 million metric tons/year (MMT/Y) (USDA, 2011), whereas orange constitute about 60% of the total production (FAO, 2004).The annual production of citrus in Egypt is about 3.233 MMT/Y (AOAD, 2009) including oranges, lemons, grapefruits and mandarins represent approximately 98% of the entire industrialized fruits, since orange being the most relevant one with approximately 82% of the total citrus fruits. About 70% of citrus amounts are manufactured to citrus products such as fresh and caned juice, jam, marmalade and syrups or citrus-based drinks. In addition, flavonoinds, essential oils and pectin are valuable by-products that are extracted from the industrial waste (Izquierdp and Sendra, 2003; El-Shenawi, 2011). As a matter of fact, the edible part of oranges is small, so large amounts of waste materials such as peels and seeds are formed during their processing which have adverse environmental impact (Manthey and Grohmann, 2001; Laufenberg et al., 2003; Montgomery 2004). On the other hand, these wastes are promising materials in food industries due to their nutritional and technological properties. It was reported that citrus seed are a valuable source for plant protein and oil (Wolfe et al., 2003). At the last decades, the demand of protein and edible oils are increased due to the growth of population and negative impact of environmental degradation on agriculture, especially in African development countries. Based on this outlook, it could be inferred the importance of scientific research, especially in poor countries, to discover nontraditional new safe and continuous food resources to fill the food gap between supply and demand for edible protein and vegetable oils. Nowadays, there are considerable researches on recovery, recycling and upgrading of citrus waste (peels and seeds) to higher values and useful products as well (Bocco et al., 1998; Fernandez-Lopez et al., 2004; Larrea et al., 2005; Reda et al., 2005; Reddy and Yang 2005; Kang et al., 2006, Li et al, 2006). However, the importance of citrus wastes are not only due the presence of essential oils, dietary fibers and pectin in their peels but also their seeds contain plant protein and oil that can be used as food ingredient of a number of products. The bitter orange (*Citrus aurantium*), although resembling the orange, it differs by several characters. Its pulp is acidic and bitter as well the albedo is thicker than orange. BOS is industrial citrus by-product which is not watchful studied; therefore this work was performed to throw some light on the chemical and functional properties of BOSF as well its in-vitro digestibility.

MATERIAL AND METHODS

Material

Bitter orange (*Citrus aurantium*) fruits were picked up from the farm of Horticulture Department, Faculty of Agriculture, Tanta University located at Seberbay, Tanta city, Al-Gharbia governorate, Egypt in October 2010. The fresh fruits were washed with tap water and then the seedswere separated, washed and dried in electric oven at 90ºC after that the dried clean seeds were milled using (Moulienx LM 25924A/701-4709-R, France) grinder. The meal was defatted with petroleum ether 60 - 80ºC in a Soxhlet apparatus and dried in an electric oven for two hours at 105ºC. The resultant was regroundby (Moulienx LM 25924A/701-4709-R, France) grinder and the powder was sieved to pass through 0.45□m sieve (60 mesh) to obtain BOCF and kept into polyethylene bags in a deep freezer at -18°C for further analysis.

Methods

Functional properties of bitter orange seed flour

Foaming capacity (FC) and foam stability of BOSF was conducted using the method of Lawhon and Cater (1971). Whipping capacity (WC) was measured according to the method of Kramer and Kwee (1977). Emulsification capacity (EC) of BOSF was also determined by the procedure described by McWatters and Cherry (1975). Water and oil holding capacities (WHC) and (OHC) were determined by the method of Sosulski et al. (1976). However least gelation concentration was determined using a modification method of Coffmann and Gercia (1977) as described by Sathe and Salunkhe (1981). Protein solubility was determined as outlined by Persons et al. (1991).

In vitro protein digestibility (IVPD)

In vitro protein digestibility of BOSF was determined by the method as described by Khalil et al. (2007). The protein was calculated according to the following equation:

$$\textbf{In vitro protein digestibility} = \frac{\textbf{\textit{Soluble protein}}}{\textbf{\textit{Total protein}}} \times \mathbf{100}$$

Determination of nitrogenous compounds

Total nitrogen (TN) of BOSF was determined using micro-Kjeldahl apparatus according to AOAC (2000) (method no. 95036). Non – protein nitrogen (NPN) content was also determined following to the method of De-Luman and Reyes (1982). Protein nitrogen (PN) content was calculated by subtract NPN from TN. Total crude protein and true protein (TP) were calculated by multiplying TN and PN in the factor 6.25, respectively.

Determination of total amino acids content

Amino acids composition of BOSF was determined according to (Bailey, 1967). Computed -protein efficiency ratio(C-PER) was calculated by the procedure of Hus et al. (1978) using amino acid composition and in vitro protein digestibility data. The chemical score also was calculated according to FAO/WHO (1990) as follows:

$$\text{Chemical Score} = \frac{mg\ per\ g\ of\ essntial\ amino\ acids\ in\ tested\ protein}{mg\ per\ g\ of\ esential\ amino\ acids\ in\ refernce\ protein} \times 100$$

RESULTS AND DISCUSSION

Functional properties

Due to the increasing in World population and the lack of animal protein, especially in the developing countries, the demand of both conventional and nonconventional plant protein increases. These alternative plant proteins must have nutritional value and good functional properties so they can be used as food ingredients.

Foaming capacity (FC) and whipping properties

Foam is a colloid of many gas bubbles trapped in a liquid. The foam can be produced by whipping air into liquid as much and fast as possible (Sikorski, 2002). Foaming capacity and foam stability of food protein are depend on some criteria including type of protein, degree of denaturation, pH and temperature of the medium as well whipping methods (Sikorski, 2002; Adebowale and Lawal, 2003).

As shown in Table (1), FC of BOSF in citrate-

Table 1. Foam capacity and stability of bitter orange seed flour.

Sample	After 30 second		After 10 mint		After 2 hours	
	pH 4	pH 7	pH 4	pH 7	pH 4	pH 7
Foam (ml)	2.6	2.0	2.3	2.0	2.3	2.0
Total Volume (ml)	52.6	52.0	52.3	52.0	52.3	52.0

Table 2. Some physicochemical properties of BOSF.

Parameter	Value (ml/g)
Emulsification Capacity (EC)	15.5
Water Holding Capacity (WHC)	2.6
Oil Holding Capacity (OHC)	2.1

phosphate buffer at pH 4 is 2.6 ml decreased to 2.0 ml in citrate- phosphate buffer at pH 7. The volume of foam was markedly decreased by elongation of retention time. Thus, foaming capacity and stability of BOSFwere depended on the acidity of the medium. So, foam capacity of BOSFwas better at lower pH than that of higher pH under the same conditions while, foam stability of BOSF was better at higher pH 7.These results are in contrast with those reported by Gafer (1995) who stated that some citrus seed flour proteins were improved in the alkaline region compared with the acidic one.

Emulsification capacity, water and oil holding capacity

Emulsification capacity (EC)

EC of protein-containingproducts like legume flours may result from bothsoluble and insoluble protein, as well othercomponents, such as polysaccharides. Proteincan emulsify and stabilize the emulsion by decreasingsurface tension of oil droplet and providing electrostatic repulsionon the surface of the oil droplet (Sikorski, 2002). While some types of polysaccharides can helpstabilize the emulsion by increasing the viscosityof the system (Dickinson, 1994).Emulsion is unstable colloid system and thus need energy input the system through shaking, stirring, homogenizing, or spraying to form stable emulsion. As shown in Table (2) emulsion of BOSF is 15.5 ml/g which is much higher than those undefatted 2.0ml/g and defatted pumpkin seed flours 1.8 ml/g(Cuthbertson, 1989), orange seed flours (5.0 and 4.0 ml/g)(Akpata and Akubor1999). The high emulsion activity and stability suggest the possible use of BOSFin processed meat products like sausagesand in stabilizing colloidal food systems.

Water Holding Capacity (WHC)

WHC is an index for the quantity of water which is absorbed by seed flour. Therefore, flour with high water absorption is suitable for bakery products as the hydration improve handling characteristics of the final products. As shown in Table (2), WHC of BOSF is (2.6 ml/g) higher than those of undehulling and dehulling orange seed flours (2.2 and 2.4 ml/g) as mentioned by Akpata and Akubor (1999), since dehulling process increases WHC. The increment of orange seed flour may be related to polar amino acid residues in portion as well polar groups in carbohydrates that present in the flour whereas, their affinity for binding water molecules is strong (Lawal and Adebowale, 2004; Yusuf et al., 2008).The higher water absorptivity flour may beused in the formulation of some foods such as sausage,dough's, processed cheese, soups and baked products confirmed by Olaofe et al. (1998).

Oil holding capacity (OHC)

OHC is a parameter indicates the ability of flour to entrap oil (Kinsella, 1976). The major chemical component affecting oil absorption capacity affecting OHC is protein, which involves both hydrophilic and hydrophobic amino acid residues. Therefore, the higher the hydrophobic amino acid residue in protein is, the higher the OHC.

Table (2) displayed that OHC of BOSF is 2.1 ml/g. This result is higher than that of undehulling and dehulling sweet orange seed flours (0.49-0.90 ml/g), since dehulling process increases the value of OHC (Akpata and Akubor, 1999). Also, denaturation and /or digestion of protein may bring out of the core of proteins non-polar side chains that bend hydrocarbon moieties of oil (Lawal and Adebowale, 2004). It is well known that

Table 3. The least gelation of different concentration of BOSF.

Flour % (w/v)	2	4	6	8	10	12	14	16	18	20
Gelation	-	±	±	±	+	+	+	+	+	+

(–) = Not gelled (±) = slightly gelled (+) = gelled

Table 4. Nitrogenous compounds, solubility and digestibility (%) of BOSF protein.

Parameter	%
Total nitrogen (TN)	2.87 ± 0.04
Non-protein nitrogen (NPN)	0.97 ± 0.01
Protein nitrogen (PN)	2.10 ± 0.05
Crude protein (CP) (TN×6.25)	17.90 ± 0.19
True protein (TP) (PN×6.25)	12.96 ± 0.19
Protein solubility	81.00± 1.00
Protein digestibility	75.75± 2.50

OHC is importance science oil acts as flour retainer and increases the mouth feel of foods (Aremuet al., 2007). However, the flours in the present study are potentially useful in structural interaction in food especially in flavor retention, improvement of palatability and extension of shelf life particularly in bakery or meat products where fat absorption is desired (Aremuet al., 2007).

The Least Gelation Concentration (LGC)

LGC which is defined as the lowest protein concentration at which gel remainedin the inverted tube was used as index of gelation capacity. The lower LGC, the better the gelatin ability of the protein ingredient (Akintayo et al., 1999).As shown in Table (3) a good gel is formed at 10% (w/v) of heated and defatted BOSF. These results are close that of lupine flour (Sathe and Salunkhe,1981) and both of dehulling and undehulling orange seed flours 10-12% (Akpata and Akubor, 1999) as well (Gafer, 1995). Gel formation of flour is depend mainly upon the percentage and structure of different constitute of polychemicals present in foods such as proteins, carbohydrates and lipids as well the interaction between them (Adebowale and Lawal, 2003). So BOSF could be used as thickening and gelling ingredients in the field of food processing. Protein gels are aggregation of denaturated molecules, therefore, denaturated proteins is thickening and gelling ingredient rather than the native one.

Nitrogenous compounds and protein quality

Total nitrogen content of BOSF is 2.87 and non-protein nitrogen is 0.79%. Consequently, crude protein and nitrogen protein content of BOSF are17.9%and 12.96%, respectively (Table 4). Theses result is lower than those reported by Arriola-guevara et al. (2006) who pointed out that the seed of Mexican lime contained 21% proteins.

Protein quality is major determinant whether the dietary protein intake can furnish an adequate level of satisfy its function in the body. Therefore, solubility, digestibility and amino acids composition of protein are important criteria to evaluate the protein quality. The results of Table (4) display that protein solubility of BOSF is 81%. This result is higher than that of raw (22.63%) and germinated (70.70%) chickpea (Khdttak et al. 2008). Protein solubility of different seeds and legumes is improved in the alkaline medium (Sathe and Salunkhe, 1981; Idouraine et al., 1991; Osman, 2004). Digestibility of protein is an index for the nutritional value of proteins present in seed flour. Where, In Vitro protein digestibility could be attributed to good and high concentration of essential amino acids. In addition, low level of antinutritional factors such as the amounts of trypsin inhibitor, tannins and phytic acid. The results display that digestibility of BOSF is 75.75%.

Amino acid

Studies on the utilization of vegetable proteins continue to gain attention due to the worldwide increasing demand for cheap and acceptable dietary protein, particularly for the low-income nations. The need to search for unconventional legumes and oil seeds therefore attract research in this direction (Onweluzo et al., 1994; Chau and Cheung, 1998; Ezeagu et al., 2002; Horii et al., 2007; Lau and Sun, 2009). The nutritional quality of protein is principally governed by its amino acids composition. Amino acids content of BOSF is presented in Table (5).

Table 5. Amino acid composition of BOSF (g/100g protein).

Amino acid	Sample	FAO	Chemical score
Essential amino acid			
Lysine	2.38	5.8	48.8
Methoinine+ cysteine	2.4	2.5	96.0
Threonine	1.99	1.4	142.1
Isoleucine	2.16	2.8	77.1
Leucine	5.71	6.6	86.5
Valine	4.06	3.5	116.0
Phenylalanine+ tyrosine	11.08	6.3	175.9
Tryptophan	6.85	-	-
Histidine	3.72	1.9	195.8
Sum of essential	**40.53**		
Non-Essential amino acid			
Aspartic	7.08		
Proline	2.25		
Serine	3.58		
Glutamic acid	16.9		
Glycine	12.2		
Alanine	1.76		
Arginine	4.38		
Sum of non-essential	**48.12**		
1st limiting amino acid	Lysine = 48.8		
2nd limiting amino acid	Isoleucine = 77.1		
Protein efficiency ratio	1.1		

The data reveal that the minor essential amino acids (less than 5%) are Threonine (1.99) followed by Isoleucine (2.16),While, the major essential amino acids (more than 10 %) are Phenylalanine+ tyrosine (11.08). Essential Amino acids contents are further used to calculate certain nutritional parameters. The chemical score estimates the protein quality of a food. The first limiting amino acid isLysine48.8%, while the second limiting amino acid isoleucine 77.1% comparing with reference protein of FAO (1985). The protein efficiency ratio (PER) is another means of measuring a food's protein quality. The BOSF protein efficiency ratio was 1.1.

CONCLUSION

Results showed that BOSF could be used as a good source of plant protein due to its high nutritional value and functional characteristics. As the proteins of BOSF have significant solubility and digestibility as well it is containing the essential amino acids at reasonable rates. Add to that proteins present in BOSF have upright foam capacity and stability, OHC and gelation power that permit the utilization of BOSF in the field of bakery products. Also, the high WHC and emulsion activity of BOSF make it as one of the better filling materials in the field of meat processing.

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