Menoufia J. Food & Dairy Sci., Vol. 4 June (2019): 57 - 71

PRODUCTION AND EVALUATION OF SWEET WHEY DOUM BEVERAGES

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Received: Jan. 23 , 2019 Accepted: Mar. 14, 2019

ABSTRACT: *The present study was carried out to prepare whey doum beverage by soaking the crushed doum fruit in sweet whey at a ratio of 1:5 (w/v) for 4, 8 and 12 h. The physico-chemical properties, total phenolic content (TPC), total flavonoid content (TFC), antioxidant activity (AA %) were determined by HPLC, the microbial characteristics were evaluated on fresh beverage and after one week of cooled storage. The beverage samples were evaluated for their sensory characteristics appearance, taste, flavor and overall acceptability by a 15 trained panelists. Results concluded that whey could be successful for the development of whey based doum beverages with optimum sensory characteristics. Fresh sample had the best results in most evaluated parameters like TPC, TFC and AA. Moreover have excellent appearance, color, odour, taste and overall acceptability.*

Key words: *Doum, sweet whey, total phenolic content, extracts, antioxidant activity, colour index.*

INTRODUCTION

Most consumers are looking for methods to Improve the health whether it's changing food, lifestyle. Whey is a rich source like biologically active ingredients or worthy organic supplement gained from nature's resources e.g. nutritious protein source which is a high-quality that provides most essential amino acids necessary for good health (Kimball & Jefferson, 2001and Layman, 2002).

Doum fruit, (*Hyphaene thebaica*) is a desert palm growing wild over the drier areas in Egypt, Sub-Saharan of Africa, and west India. It considered as beneficial plants in the world (Fletcher, 1997). Doum usually consumed as beverages in traditional places in Egypt. It was reported that the drink has been prepared from the fruits by infusing the dried ground fruit pulp in hot water Hashem (1994). This drink is widely consumed as a health tonic as a remedy for hypertension. Doum fruits are relatively rich in protein. Its content of essential minerals which may exceed the recommended daily allowance (RDA) (Cook *et al*., 2000). Doum was reported to lower the blood pressure, when its biological activity was evaluated in rat feeding experiments (Sharaf *et al*., 1972., and Betty *et al*., 2006). Moreover, it is useful to the health and wellbeing of humankind by decreasing the gastrointestinal problems like constipation and therefore, it considered as a natural anticolon cancer (Coimbra and Jorge 2011). Doum pulp contains 4.91% proteins, 5.26% fat, 4.50% ash and 85.33% total carbohydrate (Eissa *et al*., 2008). Doum is rich in polyphenolic compounds. Its extract contains 1.20 µg/g dried plant extract as Gallic acid equivalent (GAE) and 3.70 µg/g dried

plant extract as equerafest (QE) which act to reduce the oxidative stress by scavenging free radicals (Eldahshan *et al*., 2008, Kamis, *et al*., 2003 and Jeong, *et al*., 2009). Natural antioxidant especially plant polyphenolics (Katalinic *et al*., 2006; Eldahshan *et al*., 2008, 2009) Physiologically, antioxidants play a major role in preventing the formation of free radicals, which are responsible for many oxidative processes. Antioxidants may be synthetic, such as butylated hydroxyanisole (BHA), Propyle gallate (PC) and butylated hydroxytoluene (BHT) or of natural origin such as α-tocopherol, phenolic compounds (Abas *et al*., 2006).

Doum fruit extract considered main source of phenols and flavonoids and owns significant antioxidant and anticancer activities. This is due to the high amount of their water-soluble phenolic compounds (Hsu *et al.,* 2006). The total polyphenols level are significantly affected by the kind of the extraction solvent, extraction sonicate time. However, antioxidant capacity influence by solvent extracting power (Falleh *et al*., 2012). The water extract of doum fruits can reduce hyperlipidaemia in nephrotic syndrome and leads to decrease the risk of glomerulosclerosis and atherosclerosis (Habib *et al*. 2014). The aqueous extract of *H. thebaica* is used in the treatment of bilharziasis, haematuria, and bleeding especially after child birth and as haematinic agent (Burkill, 1997).

Whey is the liquid left after milk has been coagulated and strained. It is by-product of cheese manufacturing industries. Whey contains many valuable nutrients like lactose, proteins, minerals and vitamins etc., which have crucial value as human food. It contains 45-50% of total milk solids, 70% of milk sugar (lactose), 20% of milk proteins and 70-90% of milk minerals and most importantly almost all the water soluble vitamins originally present in milk. Whey solids are not utilized beneficially and an enormous quantity of whey is being drained out globally, which poses a serious damage to environment because of its high biological oxygen demand (BOD) of 35,000 to 50,000 ppm (Ritika and Yadav 2010).

Sweet whey shows great potential for the development of dairy products due to its nutritional value, since it is not only a source of the most biologically valuable proteins, but also rich in minerals and vitamins, mainly riboflavin (Ha and Zemel, 2003). Whey contains about 50% of the milk solids together with 100% of the lactose and 20% of the protein. The lactose makes up about 75% of the total whey solids (Siso, 1996).

The main biological activities of whey proteins are suggested to include cancer prevention, increase of glutathione levels, antimicrobial function and increase of satiety response (Madureira *et al*., 2007).

The recognition of whey as a source of unique physiological and functional attributes provides opportunity for the food industry to incorporate whey and whey components into a variety of foods (National Dairy Council, 2003). Whey beverages have been recognized as a genuine thirst quencher, light, refreshing, healthful and nutritious (Prendergast, 1985). Whey based fruits beverages are more suitable for health as compared to other drinks (Sarvana Kumar, 2005). Whey and its biological components have proven its effects in treatments of cervical chronic diseases like cancer, cardiovascular, etc. From the nutrition point of view whey is too rich and it can also be used in beverages infant geriatric and atheletic food (Deveraj, 2005).

The aim of this study was to evaluate the suitability of using sweet why to produce high nutritional value fresh doum beverage.

MATERIALS AND METHODS

Material:-

Ripened and dried doum fruit (Hyphaene thebaica) were obtained from local herbal shop, Aswan, Egypt. Sweet whey was prepared from cow milk obtained from local market, Aswan, Egypt. All chemicals and solvents were of the analytical were purchased from Algomhoriya company for trading drugs, chemicals and medical instruments, Cairo, Egypt.

Methods

Preparation of doum fruit

Doum fruits were washed by tap water and then drained. The epicarp (external part) and the edible portion (mesocarp) were crushed after scraping from the seed using stainless steel knife. The crushed portion was stored at 5 ºC in a refrigerator until extraction and analysis. (Omar *et al*., 2002).

Preparation of sweet whey

Fresh cow milk was taken in required quantities in a stainless steel vessel heated to 65 ºC, rapidly cooled to 35 ºC, and rennin enzyme was added. Cheese was separated from whey using muslin cloth and sweet whey was collected, (De, 1991).

Chemical analyses of doum fruit and whey

Doum fruit or whey samples were analyzed for moisture, protein (% N × 6.25), fat, ash, total soluble solids (TSS), pH and total acidity carbohydrate according to the methods described in A.O.A.C (2010). Replicate experimental were carried out and the date were calculated on dry weight.

Preparation of doum fruit extract by sweet whey

The method of extraction was used for preparing the sweet whey extract of doum fruits presented in (Fig. 1). The method was carried out by soaking the crushed doum fruits in sweet whey at a ratio of 1:5 (w/v) for 4, 8 and 12 h. Extract was drained through one layer of cheese cloth and pressed by hand until obtaining the free running extract, then the obtained extract was filtered through a piece of cotton to remove fine particles. The TSS of the three extracts obtained was increased to reach 15% by commercial sugar. The prepared samples were divided to two parts theist part was evaluated by the trained panelist as fresh the second part was stored at 4ºC for one week then evaluated sensory and chemically proprieties such as mentioned by Aamer (2016) with some modifications.

The content of total soluble solids (T.S.S) at 20 ºC expressed as Brix was determined using a digital refractometer (2WAJ Abbe Bench Refractometer Optika Italy) as described in A.O.A.C. (2010). The pH values were measured using a digital pH-meter (JENWAY Bench top Digital with microprocessor pH meter Model :3510 (England) as described in A.O.A.C. (2010). Total acidity as % tartaric acid was determined according to A.O.A.C. (2010).

Determination of total phenol and total flavonoid contents in whey doum beverage

The sample was mixed with methanol at a concentration of 1mg/mL for determination of total phenolics content and total flavonoids content. Total phenolics content were determined by a method using Foline Ciocalteu's phenol reagent with gallic acid as a standard (Julkunen-Titto, 1985) and expressed as mg gallic acid equivalent (GAE)/g of dried extract. Total flavonoids content were determined by a method using $AlCl_3$, H_2O solution (10 g/100mL) with (p)-catechin as a standard (Shyu *et al*., 2009) and expressed as mg catechin equivalent (CE)/g of dried extract.

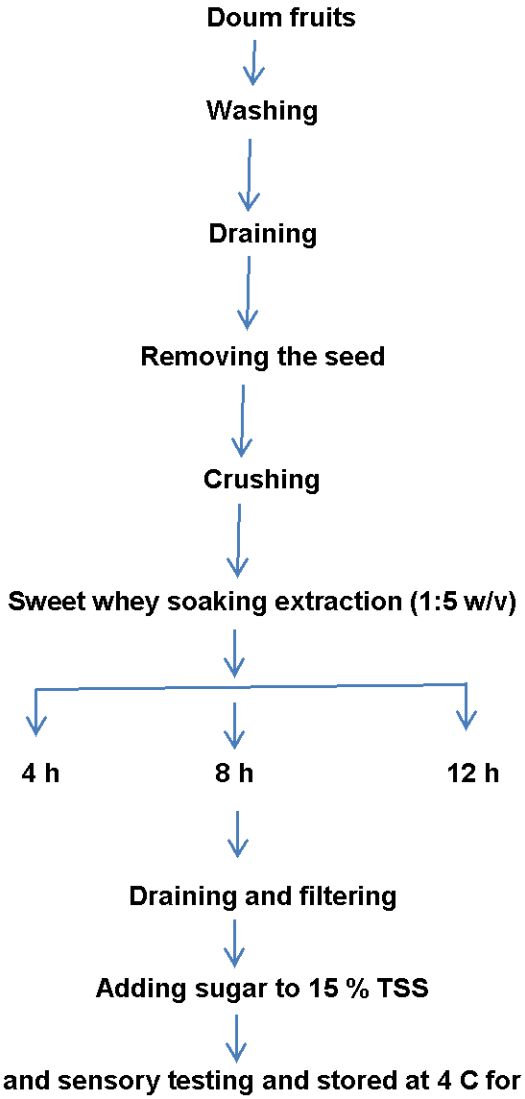

Fig. (1) Flow sheet for preparing doum fruit extract by sweet whey

Determination of antioxidant activity in whey doum beverage

The DPPH (2.2-diphenyl-1-picrylhydrazydl) radical scavenging activity of methanolic extracts was determined following the method reported by Tepe *et al* (2005).

Sensory evaluation of whey doum beverage

The beverage samples were evaluated as described by Djuric *et al.*, (2004) for sensory characteristics appearance, taste, flavor and overall acceptability by a trained panels comprising of 15 panelists drawn from faculty members and post graduate students of the food science and technology department.

Microbial Analysis:

Microbial analysis were carried out by taking 10 ml representative samples and aseptically mixed with 90 ml distilled water and homogenized by shaking. Subsequent decimal dilutions were prepared with the same diluents and in all dilutions duplicate-counting plates

were prepared of appropriate dilutions (Harrigan and Mac-Cance, 1976). Total viable count was carried out using the pour plate method (Harrigan, 1998).

Statistical analysis

The results were analyzed in statistical analyzes of Statistical Analysis System (SAS) (1999). Duncan's was used at a level of 5% of importance for comparing methods as for Snedecor and Cochran (1980).

RESULTS AND DISCUSSION

Analysis of doum fruit and whey:

Chemical characteristics of doum fruit and sweet whey are presented in Table (1). It clearly indicates that doum fruit was rich in carbohydrate and fibre. Also sweet whey was recorded 6.59, 0.30 and 4.6 T.S.S, acidity and PH, respectively. The results obtained are in agreement with the earlier studies (Wazir, 1999; Ingale *et al*., 2009; Divya *et al*., 2014 and Yonis *et al*., 2014). SAS

Physico-chemical properties of fresh whey doum beverage as affected by storage for a week

The physicochemical characteristics of whey doum beverage (fresh and after one week at 4 ºC) were evaluated. The results are recorded in Table (2).

Sample 12 (fresh) recorded the highest value of T.S.S (15.94) followed by 8 (fresh) (extracted for 8 hours fresh) then sample 4 (fresh) which recorded 15.02 and 14.83, respectively. We found no significant difference (P≤ 0.05) between all samples in T.S.S content. On the other hand after one week of storage in refrigerator we found a little increase in T.S.S content to 16.27, 16.50 and 16.69 in samples 4, 8 and 12 after one week, respectively. While titratable acidity was increased through storage after one week in refrigerator from 0.34% to 2.63%. pH value recorded some decrease after one week storage from 4.50 to 2.23. While the colour index in fresh beverage recorded 1.02, 1.07 and 1.09 in samples 4 (fresh), 8 (fresh) and 12 (fresh), respectively. Obtained data showed a little increase in colour index by storage time to reach 1.92, 1.90 and 1.89, respectively.

Table (1): Chemical composition of doum fruit (on dry weight) and sweet whey

Parameter	Doum	Sweet whey
Moisture %	3.52	92.84
Protein%	3.71	0.90
Fat%	0.84	0.34
Ash%	5.97	-
Fibre%	28.83	-
*Carbohydrate	89.46	-
T.S.S%	-	6.59
Acidity%	-	0.30
PH	-	4.6

*Calculated by difference.

Table (2): Physico-chemical properties of fresh whey doum beverage as affected by storage at 4 ºC for 7 days

	Sample	T.S.S.% Brix	Titratable acidity (%)	pH value	Colour index (420 nm)
Fresh	Extracted for 4 hours	14.83^{b}	0.33^{b}	4.50^{a}	1.02^{b}
	Extracted for 8 hours	15.02^{ab}	0.34^{b}	4.33^{a}	1.07^{c}
	Extracted for 12 hours	15.94^{ab}	0.34^{b}	4.46^{a}	1.09^{c}
Stored for one week at 4 C	Extracted for 4 hours	16.27^{ab}	2.62^{a}	3.50^{b}	1.92^{a}
	Extracted for 8 hours	16.50^{ab}	2.63^{a}	2.76^{c}	1.90^{a}
	Extracted for 12 hours	16.69^{a}	2.56^{a}	2.23^{d}	1.89^{a}

*The means in the different letters on the same column explain statistically significant differences (P≤0.05).

Total phenolics (TPC), flavonoids contents (TFC) and antioxidant activity (AA)

Table (3) shows the changes in total phenolics content (TPC), total flavonoids content (TFC) and antioxidant activity (AA) of fresh and cooled stored whey doum beverage. In general, increasing soaking time led to an increase in the TPC, TFC and AA whereas cold storage decreased these parameters.

Increasing the time of soaking raised the TPC, TFC and AA % from 7.58 (mg/100 g), 1.71 (mg/100 g) and 79.65 (%) after 4 h to 12.19 (mg/100 g), 3.78 (mg/100 g) and 90.32% after 12 h, respectively. On the other hand, cold storage caused a reduction in TPC, TFC and AA during 7 days of cold storage. These results may be due to degradation in phenolics, flavonoids content and antioxidant activity through storage. Meanwhile, decreases in phenolic compounds content did not lead systematically to a decrease of the antioxidant activity. In fact, the degradation products of phenolic compounds can also have an antioxidant activity sometimes higher than the initial phenolic compounds (Buchner *et al.*, 2006; Murakami *et al.*, 2004). While in other cases, the antioxidant activity was reduced. In general, the highest amount of TPC and TFC was extracted using soaking procedure after 12 h and cooled storage in the same sample.

Accordingly, the highest AA was recorded at the same conditions. The results indicated that increasing time of soaking enhances the release of polyphenol and flavonoid constituents from the cell wall (Makris and Rossiter 2000) and Buchner *et al.*, 2006 mentioned that flavonoids in aqueous solutions show different sensitivity to heat treatment depending on their structures.

Statistically, the data in Table (3) indicates that significant (P ≤ 0.05) difference in TPC, TFC and AA values was found among all soaking treatments for different time (4, 8 and 12 h). It is well known that plant phenolic compounds are highly effective free radical scavengers. The AA increased with the increase in concentration and the consumption of doum plant which would exert several beneficial effects by the value of its antioxidant and antimicrobial

activities (Mohamed *et al*., 2010). There are various factors such temperature, solvent extracting power, extraction time, and extraction method which significantly affect the composition of the extract (Ksouri *et al*., 2009; Aboshora *et al*., 2014). Decreases in phenol content do not lead systematically to a decrease of the AA. Indeed, the degradation products of phenolic compounds can also have an antioxidant activity sometimes higher than their initial phenolic compounds (Buchner *et al*., 2006; Murakami *et al*., 2004). Also, Aboshora *et al.* (2014) found that doum fruit extracts contain high levels of phenols and flavonoids, and possess significant antioxidant and antibacterial activities. It is evident from these findings that doum fruit can serve as a potential source of natural antioxidants and antibacterial agents, which can help to prevent diseases related to oxidative stress and pathogenic bacteria.

The proton radical scavenging action is known as an important mechanism of antioxidants. DPPH is usually used as a substrate to evaluate the antioxidative activity of natural antioxidants because it is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule (Soares *et al.,* 1997). The effect of antioxidants on DPPH radical scavenging was thought to result from their hydrogen donating ability (Shimada *et al.,* 1992). The decrease in absorbance of the DPPH radical caused by reacting between antioxidant molecules and the radical, progresses, which results in the scavenging of the radical by hydrogen donation.

The scavenging effects of whey extract from our plant (H. thebaica) on DPPH radicals increased with soaking time (Table 1). Phenolic compounds of the H. thebaica extract are probably involved in their antiradical activity (Hsu *et al.,* 2006). Sample 12 (fresh) may be viable source of bioactive compounds with better activities after fractionation.

Table (3): Total phenolic (TPC), flavonoid contents (TFC) and antioxidantn activity (AA) of fresh whey doum beverage as affected by storage time at 4 ºC.

Sample		*TPC (mg/100 g)	**TFC (mg/100 g)	***AA (%)
Fresh	Extracted for 4 hours	7.58^{c}	1.71^{e}	79.65^{d}
	Extracted for 8 hours	9.70^{b}	2.44^{c}	86.83^{bc}
	Extracted for 12 hours	12.19^{a}	3.78^{a}	90.32^{a}
Stored for on week at 4C	Extracted for 4 hours	5.09^{d}	1.09^{f}	76.44^{e}
	Extracted for 8 hours	7.69^{c}	1.99^{d}	86.08^{c}
	Extracted for 12 hours	10.13^{b}	2.89^{b}	87.80^{b}

*TPC: Total phenolic content (mg/100 g tannic acid equivalent).

**TFC: Total flavonoids content (mg/100 g rutin equivalent).

***AA: Antioxidant activity (%).

****Means with different letters on the same column explain statistically significant differences at P≤0.05.

Sensory evaluation of fresh whey doum beverage:

Data in Table (4) show the average sensory scores of different quality attributes and overall acceptability for fresh whey doum beverage. It was observed that sample 12 (fresh) was recorded highest (7.62) overall acceptability scores and for other parameters like appearance (8.00), colour (7.62), odour (7.62); and taste (7.62). So the sample 12 (fresh) was organoleptically better than other samples.

After one weak of storage at 4℃ all samples not acceptable by the panelist due to the increasing of acidity and change of odour.

Microbial Analysis

The beverage samples were analysis periodically for total plate count. The data obtained with respect to microbial load are summarized in Table (5). Total count of bacteria increases and after the completion of (7 days) storage it reaches to 3.9×10^5, 8.0×10^4 and 3.4×10^5 in the beverage samples 4 (after one week), 8 (after one week) and 12 (after one week) respectively (Figs. 2 to 7). Similar results of total plate count were reported for whey-based mango beverage (Ismail *et al.,* 2011). In spite of the potential benefits offered by fruit juices, concerns over their safety and quality have been raised; as freshly prepared juices have no process or steps to minimize the microorganisms if they are contaminated (Mahale *et al.,* 2008).

Table (4): Sensory evaluation of fresh whey doum beverage.

Fresh sample	Appearance	Colour	Odour	Taste	Overall acceptability
Extracted for 4 hours	6.87^a	6.50^a	6.37^a	6.25^a	6.62^a
Extracted for 8 hours	7.62^a	7.50^a	7.37^a	7.25^a	7.37^a
Extracted for 12 hours	8.00^a	7.62^a	7.62^a	7.62^a	7.62^a

*Means with different letters on the same column explain statistically significant differences at P≤0.05.

Table (5): Total plate Count of fresh whey doum beverage as affected by time.

Sample		Total plate Count
Fresh	Extracted for 4 hours	$2.24^d \times 10^7$
	Extracted for 8 hours	$6.0^b \times 10^6$
	Extracted for 12 hours	$2.46^d \times 10^7$
Stored for on week at 4C	Extracted for 4 hours	$3.9^c \times 10^5$
	Extracted for 8 hours	$8.0^a \times 10^4$
	Extracted for 12 hours	$3.4^c \times 10^5$

*Means with different letters on the same column explain statistically significant differences at P≤0.05.

Fig. (2): Fresh whey doum beverage at a ratio of 1:5 (w/v) extracted for 4 h.

Fig. (3): Storage whey doum beverage (for one week) at a ratio of 1:5 (w/v) extracted for 4 h.

Fig. (4): Fresh whey doum beverage at a ratio of 1:5 (w/v) extracted for 8 h.

Fig. (5): Storage whey doum beverage (for one week) at a ratio of 1:5 (w/v) extracted for 8 h.

Fig. (6): Fresh whey doum beverage at a ratio of 1:5 (w/v) extracted for 12 h.

Fig. (7): Storage whey doum beverage (for one week) at a ratio of 1:5 (w/v) extracted for 12 h.

Conclusion:

Whey can be used successful for the development of whey based doum beverages with acceptable sensory characteristics. Extracted for 12 hour fresh sample had the best results in most evaluated parameters such as Phenolics, flavonoids content and antioxidant activity. Moreover samples have excellent appearance, color, odour, taste and overall acceptability, which mean that doum powder covered unpleasant taste of whey very successfully.

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الملخص العربى

أجريت هذه الدراسة بهدف دراسة امكانية استخدام الشرش الحلو لانتاج مشروب دوم طازج عالى القيمة الغذائية عن طريق نقع مسحوق الدوم فى شرش اللبن بنسبة ١: ٥ (وزن / حجم) لمدة ٤ ، ٨، ١٢ ساعة. تم تقدير الخواص الطبيعية والكيميائية والمحتوى من المواد الفينولية والفلافونيد وكذلك النشاط المضاد للأكسدة فى المستخلص الناتج علاوة على تقدير المحتوى الميكروبى فى المشروب (الطازج والمخزن لمدة أسبوع فى الثلاجة)، أيضا تم تقييم الخواص الحسية للمشروبات بواسطة مجموعة من المحكمين لمعرفة مدى القابلية للاستهلاك من حيث (اللون ، والشكل العام، الطعم ، المذاق، الرائحة) بواسطة ١٥ محكم. وقد أشارت النتائج المتحصل عليها أن العينة الطازجة قد سجلت أفضل النتائج في معظم التقديرات التي تم تقييمها مثل الفينولات ، الفلافونويد والنشاط المضاد للأكسدة. وعلاوة على ذلك لديها مظهر ممتاز فى اللون ، والشكل العام، الطعم ، المذاق، الرائحة.

الكلمات :الدوم – شرش اللبن الحلو– محتوى الفينولات الكلية– المستخلصات– النشاط المضاد للأكسدة– دليل اللون.