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The Use of *Vitex agnus-castus* to Produce Functional Stirred Yoghurt

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ABSTRACT

Stirred yoghurt was prepared from cow's milk supplemented with different levels (0.5, 1.0, 1.5 and 2.0% w/v) of Vitex agnus-castus (VAC). The chemical composition, rheological properties, microbiological quality and sensorial attributes were evaluated in fresh and cold stored product. The addition of VAC increased total solids, fat, ash, TVFA, Ca, Mg, Fe and pH of the product. The addition of VAC had no effect on protein content and acidity, while it increased significantly the apparent viscosity and water holding capacity (WHC) of the prepared fresh and stored yoghurt. Total bacterial count (TBC) and count of yeasts and moulds (YMC) were almost less in the fresh and stored VAC supplemented samples. The taste of plant appeared clearly in the fresh yoghurt, whereas it disappeared by advancing storage period to give pleasant and accepted product.

Keywords: Vitex agnus-castus, Composition, Quality, Stirred yoghurt.

INTRODUCTION

It is well-known that yoghurt is recognised as a nutritious and healthy fermented dairy product in Egypt as well as in different regions in the world. In addition, yoghurt and its related products have recently a growing area of interest based on their concenience and ability to deliver more nutritional and health benefits via some additives and some other live micro-organisms (Allgeyer *et al*., 2010; Granato *et al*., 2010 ; Han *et al*., 2012 ; Freitas *et al*., 2015 and Fazilah *et al*., 2018). It protects the body from under and over nutrition, including musckeletal impairment, immunosencence, cardiometabolic diseases, and cognitive impairment (El-Abbadi *et al*., 2014). Consumption of yoghurt has a positive effect on the lipid profile in plasma of healthy women (Elmadfa, 2006).

Vitex agnus castus (VAC) is deciduous shrub that is native to Mediterranean countries and central Asia. The popularity of this plant is mainly due to using it in female disorders especially disorders linked with female reproductive system (Vimal *et al*., 2011) and in general for its pharmaceutical and health benefits. It contains dopaminergic compounds which are important for improving premenstrual syndrome (Mayo and Facog, 1998 ; Wuttke *et al*., 2003 and Daniete *et al*., 2005). It has exhibited prgesteronic and estrogenic properties, so we should avoid it during pregnancy (Niroumand *et al*., 2018).

VAC is used in many diseases as antiandrogenic, anti-inflammatory, analgesic, hepatoprotective and anxiolytic because it contains vitex, nishindaside and negundoside (Vimal *et al*., 2011 ; Kuruuzum-Uz *et al.* 2008). It effective also for psychological symptoms (Atmaca *et al*., 2003). This plant is rich in flavonoid derivatives, which have high antioxidant (Kuruuzum-Uz *et al*., 2008).

VAC contains a variety of potential bioactive molecules, such as iridoids, flavonoids, diterpeoids, derivatives and phytosterodids which are anti-inflammatory, antihistaminic, antioxidant, hepatoprotective, antihistaminic and antihistaminic properties (Meena *et al.*, 2011). Oil of this plant has an important role for antibacterial activities and anti-fungal activities (Ghannadi *et al.*, 2012 and Asdadi *et al.*, 2014).

The German commission E recommends 30-40 mg of dried fruit extract daily from VAC (Niroumand *et al.*, 2018).

Therefore, the objective of the present work was to produce a healthy yoghurt for women since it was reported as mentioned before that VAC has a significant medical role as antiandrogenic, anti-inflammatory, analgesic and hepatoprotective as well as it has exhibited progesteronic and estrogenic properties. Impact of VAC on composition, properties and quality of the prepared stirred yoghurt was taken into consideration.

MATERIALS AND METHODS

Materials:

Fresh cow's milk was obtained from the herd of Sakha Experimental Station. The chemical composition of standardized milk (4% fat) used to make stirred yoghurt was fat 4%, total protein 3.51%, ash 0.72%, total solids 12.23%, whereas its acidity was 0.18% and pH was 6.5.

Yoghurt starter culture consisting of *Streptococcus thermophilus* & *Lactobacillus delbrueeckii* sub ssp. bulgaricus (DVS) was obtained from Chr. Hansen's Laboratories Copenhagen, Denmark. Gelatin was obtained from Misr Food additives Comp. (MIFAD), Cairo.

The flowering stems (flower, leaves and twigs) of VAC were obtained from El-Sadat city, Menofyia

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Governorate, Egypt. The fruits were collected from the tree early in the morning at the end of the flowering stage that took place in November.

Methods:

Preparation of VAC:

The collected samples (mainly the fruiting tops and leaves) were air dried in a ventilated oven at 45^{o}C then the grinding was done by the mill and kept in paper bags. The chemical composition of VAC used in making yoghurt was TP 16.95%, fat 4.07%, fiber 31.5% and ash 8.95%.

Stirred yoghurt manufacture :

Stirred yoghurt was made from cow's milk as mainly described by Tamime and Robinson (1999). Milk samples were heated at 90^{o}C for 10 min, 0.5% gelatin and 5% sugar were added and then cooled to 42^{o}C (control) and to 60^{o}C for VAC addition at the ratio of 0.5, 1.0, 1.5 and 2.0% (A1, A2, A3 and A4) and then cooled to 42^{o}C. Milk samples from different treatments were inoculated with yoghurt starter culture and incubated at the same temperature to rich pH ~4.6. The prepared samples were stirred before filling into 100 ml plastic cups and then cooled to 4^{o}C. Three replicates were done from each treatment. The resultant yoghurt samples were stored at 4^{o}C for 15 days.

Chemical and physicochemical analysis:

Total protein, ash, fat contents and titratable acidity (as lactic acid) were determined according to AOAC (2005). The pH value was measured using pocket pH meter (I Scientific, USA, Model Ia_{125}). Ca, Co, Ni, Mg, Fe and Zn were determined by atomic absorption according to the method mentioned by Sparks (1996). TVFA content was determined by a direct distillation method according to Kossikowski (1978). The results were expressed as ml 0.1 N NaOH/100 g stirred yoghurt.

Viscosity and water holding capacity (WHC):

The apparent viscosity of the mixtures was evaluated by means of a digital Brook Field Viscometer (Model LVDV-E), Brook Field Engineering Laboratories, (Inc. USA), using ultra low adapter (ULA) unit altached to the viscometer as described by Khalifa and Ghanimah (2013). Samples were tested in triplicate at 5±1^{o}C with spindle No. 64. The viscosity reading was recorded as centipoises (CP).

The water holding capacity (WHC) of yoghurt was measured by centrifugation of five gram yoghurt sample at 4500 rpm for 30 min at 10^{o}C (Jouan, MR 1822, France). The WHC was calculated (Isanga and Zhang, 2009) as follows:

$$\textbf{WHC (\%)} = 1 - (W1 / W2) \times 100$$

Where, **W1 = weight of whey after centrifugation**

W2 =- yoghurt samples weight

Microbiological analysis:

Total bacterial count (TBC):

It was determined according to Difico manual (1984), using tryptone Glucose Extract Agar medium (TGEA). The plates were incubated at 32^{o}C for 2-3 days.

Yeasts and moulds count (YMC):

Count of yeasts and moulds was determined according to Luck and Govorn (1990) using potato dextrose agar (PDA) medium.

Sensory evaluation:

The organoleptic properties of the prepared stirred yoghurt were mainly assessed according to the sensory scheme given by El-Shibiny *et al*. (1979). In this respect, 40 points were given for body and texture including general appearance (10 points), viscosity (10 points), smoothness (10 points) and wheying-off (10 points). The flavour attributes (60 points) were evaluated taking into consideration giving the maximum attainable score of 10 points for each of acid, bitterness, flat, foreign, cocked and unclean. The fresh and stored samples were evaluated by ten panelists.

Statistical analysis:

All measurements were done in triplicates and data were reported as meas±SE. Analysis of variance and Duncan's test were carried out according to SPSS computer program (SPSS, 1999)

RESULTS AND DISCUSSION

Table (1) shows that increasing the amount of VAC added increased significantly ($P \leq 0.05$) the TS content. Such increase of TS may be due to the amount of fiber in VAC. The total protein was not affected by adding the plant ($P > 0.05$). However, the more fat in stirred yoghurt at high concentration of plant added may be due to release of oil at high concentration. The plant has many minerals like calcium and zinc (El-Abbadi *et al.*, 2014). So, concentration of ash in stirred yoghurt increased by increasing plant concentration in the prepared yoghurt. The results of TVFA in the VAC treated samples increased significantly, the highest results were at the highest VAC concentration. VAC contains linoleic acid, which can bind to estrogen receptors and induce certain estrogen incurable genes (Liu *et al.*, 2004). VAC rich also with sabinene, 1, 8 – cineole and α-pinene (Stojkovic *et al.*, 2011).

It may be of interest to note that the prementioned changes in the chemical composition of stirred yoghurt as affected by the addition of VAC were recorded also in the stored yoghurt samples. However, the noticed changes – on storage – could be attributed to loss of moisture with advancing storage which increased TS, protein, fat and ash contents of the product, while more TVFA were liberated in the stored samples. Such changes were recorded with different rates in the untreated (control) and VAC-treated samples.

Table (2) indicates that the addition of VAC increased minerals content of stirred yoghurt. Increasing the amount of VAC increased Ca, Mg, Fe and Zn content of the product but there were no changes in Co and Ni. Such increases of minerals agrees with the trend of data given in Table (1) for ash content. Richness of VAC with minerals was responsible for the recorded data of yoghurt. Ozkaya *et al*. (2013) gave more details about elements content of VAC.

Table 1. Changes in total solids, total protein, fat, ash and TVFA (ml 0.1 N NaOH/100 g) of stirred yoghurt when fresh and at 7 and 15 days of cold storage as affected by VAC (%) (Average±SE of 3 replicates).

Storage period (days)	C	A1	A2	A3	A4
Total solids (%)					
Fresh	12.65±0.08^{e}	12.92±0.15^{d}	13.26±0.05^{c}	13.42±0.20^{b}	13.53±0.09^{a}
7	13.23±0.14^{e}	13.40±0.09^{d}	13.49±0.09^{c}	13.65±0.08^{b}	13.89±0.12^{a}
15	13.41±0.20^{e}	13.68±0.12^{d}	13.54±0.12^{c}	13.70±0.08^{b}	14.08±0.16^{a}
Total protein (%)					
Fresh	5.12±0.07^{a}	5.09±0.20^{a}	5.14±0.12^{a}	5.14±0.17^{a}	5.15±0.08^{a}
7	5.23±0.12^{a}	5.24±0.26^{a}	5.22±0.14^{a}	5.28±0.20^{a}	5.28±0.27^{a}
15	5.69±0.17^{a}	5.62±0.18^{a}	5.65±0.18^{a}	5.65±0.30^{a}	5.87±0.13^{a}
Fat (%)					
Fresh	3.12±0.03^{c}	3.13±0.02^{c}	3.31±0.05^{c}	3.42±0.07^{b}	3.61±0.04^{a}
7	3.14±0.05^{d}	3.17±0.06^{c}	3.28±0.01^{c}	3.44±0.02^{b}	3.62±0.81^{a}
15	3.23±0.04^{d}	3.22±0.05^{d}	3.40±0.05^{c}	3.51±0.09^{b}	3.70±0.12^{a}
Ash (%)					
Fresh	0.67±0.08^{d}	0.69±0.03^{c}	0.70±0.01^{c}	0.73±0.09^{b}	0.75±0.10^{a}
7	0.68±0.07^{d}	0.72±0.02^{c}	0.73±0.06^{c}	0.75±0.43^{b}	0.78±0.03^{a}
15	0.70±0.04^{d}	0.74±0.04^{c}	0.76±0.08^{b}	0.80±0.07^{a}	0.80±0.08^{a}
TVFA (ml 0.1 N NaOH/100 g)					
Fresh	0.17±0.12^{d}	0.275±0.07^{c}	0.333±0.09^{b}	0.333±0.12^{b}	0.484±0.16^{a}
7	0.22±0.18^{c}	0.360±0.23^{b}	0.360±0.08^{b}	0.484±0.05^{a}	0.513±0.09^{a}
15	0.22±0.04^{d}	0.363±0.12^{c}	0.484±0.06^{a}	0.605±0.12^{a}	0.605±0.00^{a}

*** C, control and A1, A2, A3 and A4 represent 0.5, 1.0, 1.5 and 2% of VAC, respectively.**
Averages (a, b …. etc) within the same row with different superscripts differed significantly (P≤0.05).

Table 2. Effect of VAC on the Ca, Mg, Fe, Zn, Co and Ni contents of the fresh stirred yoghurt.

	C	A1	A2	A3	A4
Ca	205	221	224	230	235
Mg	18.6	21.2	24.4	29.6	33.2
Fe	0.1	0.18	0.21	0.24	0.28
Zn	0.6	0.6	0.6	0.8	0.8
Co	0.0	0.0	0.0	0.0	0.0
Ni	0.0	0.0	0.0	0.0	0.0

*** See legend to Table (1) for details.**

The pH value and acidity (%):

The results in Table (3) showed that there was some change in pH and acidity when VAC was used. It increased pH and decreased acidity by increasing VAC concentration. The values of them were insignificantly (P>0.05) different in C and A1 while a significant increase in pH and decrease in acidity were noticed at the highest VAC addition. This was true in fresh and stored samples. However, a gradual decrease in pH and increase in acidity were recorded with advancing storage. These changes seem to be affected by the amount of VAC added.

Table 3. Changes in pH and acidity of stirred yoghurt when fresh and at 7 and 15 days of cold storage as affected by VAC (%) and cold storage (Average±SE of 3 replicates).

Storage period (days)	C	A1	A2	A3	A4
pH value					
Fresh	4.73±0.21^{b}	4.76±0.08^{b}	4.82±0.09^{a}	4.85±0.09^{a}	4.90±0.09^{a}
7	4.45±0.30^{b}	4.46±0.13^{b}	4.54±0.15^{a}	4.54±0.04^{a}	4.59±0.18^{a}
15	4.28±0.18^{c}	4.30±0.14^{b}	4.32±0.21^{b}	4.38±0.11^{a}	4.43±0.20^{a}
Acidity (%)					
Fresh	0.65±0.12^{a}	0.65±0.08^{a}	0.63±0.12^{a}	0.61±0.20^{b}	0.59±0.09^{b}
7	0.69±0.18^{a}	0.70±0.13^{a}	0.68±0.09^{a}	0.68±0.14^{a}	0.66±0.04^{b}
15	0.73±0.04^{a}	0.72±0.20^{a}	0.73±0.12^{a}	0.70±0.16^{ab}	0.68±0.15^{b}

*** See legend to Table (1) for details.**

Viscosity and water holding capacity (WHC):

Table (4) shows that the apparent viscosity of all VAC supplemented yoghurt was significantly higher than the control whereas viscosity was increased with the increase in the percentage of the added VAC. Also the viscosity of yoghurt from different treatment increased with advancing storage.

Table (4) shows also that water holding capacity (WHC%) increased (P≤0.05) with increase concentration of VAC. Also, decreased with advancing storage.

The changing in viscosity and WHC may be due to increased content of total solids that proportional with the amount of VAC added. Also, the low pH should be taken into consideration in this respect. The pith cells of VAC contain starch (Dogan *et al.* 2008). The starch bind more water and increase viscosity (Tamime and Robinson, 1999).

Table 4. Changes in viscosity and water holding capacity (%) of stirred yoghurt when fresh and at 7 and 15 days of cold storage as affected by VAC (%) (Average±SE of 3 replicates).

Storage period (days)	C	A1	A2	A3	A4
Viscosity (cp)					
Fresh	1501±0.12e	1720±0.11^{d}	1805±0.31^{c}	1910±0.28^{b}	2015±0.19^{a}
7	2210±0.18e	2325±0.30^{d}	2402±0.08^{c}	2559±0.11^{b}	2809±0.21^{a}
15	2611±0.04e	3805±0.11^{d}	2921±0.19^{c}	3101±0.02^{b}	3251±0.19^{a}
WHC (%)					
Fresh	56.9±0.24^{d}	57.9±0.25^{c}	60.8±0.40^{b}	61.2±0.19^{ab}	62.9±0.28^{a}
7	55.7±0.33^{d}	56.6±0.23^{c}	58.3±0.32^{b}	59.5±0.38^{ab}	61.0±0.15^{a}
15	54.6±0.14^{d}	55.7±0.35^{c}	57.4±0.27^{b}	58.7±0.40^{a}	59.3±0.43^{a}

*** See legend to Table (1) for details.**

Total bacterial count (TBC) and counts of yeast & moulds (YMC):

Table (5) reveals TBC and YMC of fresh and stored stirred yoghurt as affected by supplementation with

VAC. Such counts were significantly ($P \leq 0.05$) decreased when VAC was used at higher concentrations (A3 and A4 treatments). This was true in fresh and stored yoghurt samples and could be due to the antimicrobial impact of VAC (Meana *et al*., 2011). A gradual increase in the counts were recorded during storage reaching the maximum counts at the end of storage period. The rate of increase was more pronounced in the control samples confirming impact of VAC as a antimicrobial agent in this respect.

Table 5. Effect of VAC on total bacterial count and yeast and moulds count (log CFU/g) of stirred yoghurt when fresh and at 7 and 15 days of cold storage (Average±SE of 3 replicates)

Storage period (days)	C	A1	A2	A3	A4
Total bacterial count					
Fresh	6.12 ± 0.11^{a}	6.09 ± 0.21^{a}	6.03 ± 0.18^{ab}	6.01 ± 0.21^{b}	5.95 ± 0.11^{b}
7	6.73 ± 0.18^{a}	6.61 ± 0.33^{ab}	6.54 ± 0.09^{b}	6.44 ± 0.08^{c}	6.31 ± 0.14^{d}
15	6.95 ± 0.06^{a}	6.81 ± 0.18^{b}	6.73 ± 0.31^{c}	6.51 ± 0.11^{d}	6.36 ± 0.20^{e}
Yeast & mould count					
Fresh	4.32 ± 0.13^{a}	4.33 ± 0.12^{a}	4.21 ± 0.09^{b}	4.03 ± 0.18^{c}	4.01 ± 0.11^{c}
7	5.11 ± 0.20^{a}	5.00 ± 0.18^{b}	4.81 ± 0.11^{c}	4.70 ± 0.15^{c}	4.52 ± 0.09^{e}
15	6.25 ± 0.16^{a}	5.95 ± 0.11^{b}	5.11 ± 0.23^{c}	4.81 ± 0.28^{d}	4.63 ± 0.17^{e}

*** See legend to Table (1) for details.**

Organoleptic properties:

Table (6) reveals that the fresh control and samples from treatments A1 and A2 had significant higher score for body and texture since much lower scores were given for treatments A3 and A4. This was also noticed in 7 and 15 days old product which could be attributed to the product supplemented with more VAC (Treatments A3 and A4) was more viscous and its appearance was not pleasant as that of the control. The control, A1 and A2 samples were characterized by smooth body and silky texture.

Table 6. Organoleptic properties of stirred yoghurt as affected by added VAC when fresh and at 7 and 15 days of cold storage. (Average±SE of 10 panelists)

	C	A1	A2	A3	A4
Fresh					
Body & texture (40)	39.30 ± 0.21^{a}	39.35 ± 0.28^{a}	38.70 ± 0.11^{a}	35.25 ± 0.41^{b}	30.10 ± 0.09^{c}
Flavour (60)	57.53 ± 0.12^{a}	57.10 ± 0.21^{a}	55.20 ± 0.21^{b}	50.20±0.31e	45.10 ± 0.12^{d}
Totalscore (100)	96.83 ± 0.09^{a}	96.45 ± 0.14^{a}	93.90 ± 0.23^{b}	85.25 ± 0.23^{c}	75.20 ± 0.13^{d}
7 days					
Body & texture (40)	38.21 ± 0.18^{a}	38.40 ± 0.03^{a}	38.55 ± 0.12^{a}	35.55 ± 0.13^{b}	35.10 ± 10.22^{b}
Flavour (60)	56.40 ± 0.11^{a}	55.30 ± 0.09^{a}	54.10 ± 0.18^{b}	55.45 ± 0.23^{a}	52.11 ± 0.11^{c}
Totalscore (100)	94.61 ± 0.18^{a}	93.70 ± 0.14^{a}	92.65 ± 0.08^{ab}	91.00 ± 0.22^{b}	87.20 ± 0.08^{c}
15 days					
Body&texture (40)	35.10 ± 0.13^{a}	36.35 ± 0.21^{a}	35.45 ± 0.24^{a}	32.10 ± 0.18^{b}	31.85 ± 0.11^{b}
Flavour (60)	54.30 ± 0.09^{a}	54.45 ± 0.13^{a}	54.50 ± 0.04^{a}	52.55 ± 0.08^{b}	50.10 ± 0.15^{b}
Totalscore (100)	89.40 ± 0.11^{a}	90.80 ± 0.18^{a}	89.95 ± 0.23^{a}	84.65 ± 0.08^{b}	81.95 ± 0.12^{c}

*** See legend to Table (1) for details.**

On the other hand, the fresh control (Table 6) ranked the maximum score for flavour, whereas a gradual and significant decrease in the scoring points were noticed with increasing the amount of VAC added. The taste of the used VAC may be responsible for the unpleasant taste of the fresh product that was rejected by some panelists. Such taste may be due to presence of flavonoids (Vitexin, casticin), iridoid glycoside (agnuside, aucubin) P-hydroxybenzoic acid, alkaloids, essential oils and fatty oils (Zsuzsanna *et al*., 2007).

In general, the scores given for the body and texture of the stored product were almost less than the feresh one, whereas much improvement in the flavour was noticed during storage. This may be due to formation of more acid and flavour components of yoghurt which overcome the taste of VAC.

In conclusion, VAC can be used successfully at the level of 1% in making stirred yoghurt. If the high concentration 2% is used for its importance, it is preferred to cold store the product to improve its flavour.

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استخدام نبات كف مريم فى تصنيع يوجورت وظيفى

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كف مريم شجيرة عطرية متساقطة الاوراق والتى تشبه اليد المفتوحه وتتميز هذه الاوراق برائحة عطرية وطعم لاذع ، ومن أهم المركبات الفعالة فى هذا النبات المركبات الفلافونيدية Flavonids والتى منها مادة الكاستسين Casticin والتى تعتبر بديلا للهرمونات الانثوية بجانب فاعلية متميزة من الناحية الطبية للعديد من الامراض الخاصة بالمرأة ، لهذا اهتم هذا البحث بدراسة تدعيم اللبن البقرى المعد لصناعة اليوجورت المقلب بنبات كف مريم فى الصورة المجففة بنسب ٠.٥ ، ١ ، ١.٥ ، ٢% (وزن/حجم). ودراسة تأثير هذا التدعيم على التركيب والخواص المختلفة لليوجورت. اظهرت نتائج تحليل اليوجورت الطازج والمخزن مبردا لمدة ٧ ، ١٥ يوما ان اضافة مسحوق كف مريم زاد من المحتوى من الجوامد الصلبة ، الدهن ، الرماد ، الاحماض الدهنية الكلية الطيارة ، الكالسيوم ، الماغنسيوم ، الحديد ، كما ارتفعت قيم الرقم الهيدروجينى وزادت اللزوجة الظاهرية وخاصية مسك الماء وكان لاضافة مسحوق كف مريم تأثيرا مثبطاً على البكتريا والخمائر والفطريات حيث قل العدد بزيادة كمية المسحوق المضافة . اوضحت نتائج التحكيم الحسى ظهور طعم كف مريم فى المنتج الطازج وكان ذلك غير مقبولا لعدد من المحكمين فى حين ان هذا الطعم لم يظهر فى اليوجورت المخزن وربما يرجع ذلك لتغلب تأثير الحموضة الناشئة ومكونات نكهة اليوجورت . وبصفة عامة كان قوام وتركيب ونكهة اليوجورت المقلب والمدعم بكف مريم والمخزن تحت تبريد مقبولا بدرجة كبيرة لدى المحكمين.