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Development of Anti-Aging Toffee by incorporating Beta Vulgaris, Punica granatum and Saccharam Officinarum and Study of its Acceptability and Stability

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Abstract

Product development has become the prime importance for food industries and societies. In the present study a new nutraceutical (anti-aging) toffee was produced by incorporating of Beta Vulgaris, Punica granatum and Saccharam Officinarum. Among the various combination, the toffee containing 100g each Beta Vulgaris and Saccharam Officinarum was found better for nutritional quality and organoleptic properties. The moisture, ash, protein, fiber, reducing sugar, water activity, deformation and energy content of newly developed anti-aging toffee were found to be 11.48±0.06%, 0.80±0.01%, 0.60 ± 0.03%, 1.9 ± 0.03%, 12.6±0.62%, 0.56(Aw), 4.35±0.5N and 85.7±2.1kcal/100g, respectively. On the sensory and microbiological point of view, the presently developed anti-aging toffee was found highly acceptable up to 4 months. The total yeast, aerobic plate count and mold count were found to be the acceptable according to food standards. After 4 months, the microbial counts were increased and deterioration in the product quality was observed. The calorific values indicate that the newly developed anti-aging toffee contains only 4.3% of the daily calories needs of a senior citizen. The newly established toffee imparts anti-aging impact on the aged people due to medicinal importance of the ingredients. There are several confectionery products sold in the market and few of them have medicinal properties, but no toffee with anti-aging properties has been reported in the literature earlier. Therefore, the introduction of this first ever anti-aging toffee in the market will improve the health of the elderly people as well as the product cycle of the industry. It also has the market competent price therefor the product is likely to gain the attraction of consumer and improves the market trends.

Keywords: New food product development, toffee, anti-aging, beta vulgaris, saccharam officinarum

Introduction

Fruit and vegetable containing functional foods are considered very important due to their effects on health (Ninfali and Angelino 2013). Progressive deterioration of physiological functions makes skin cell turnover slows down and the dermal structure begins to collapse. As a result of dermal aging the wrinkles and dry lines are appeared on skin (Ammar, Ghorab *et al.* 2016).

Beta Vulgaris and *Saccharam Officinarum* has high levels of antioxidants with efficient antiaging properties. *Beta Vulgaris* contains anti-free radical vitamins (A, C, folate vitamin B-9). Vitamin A reverses age-related skin thinning stimulate, renewal of skin collagen and reversal of uneven skin pigmentation. Vitamin C helps to reverse uneven skin pigment rebuild skin collagen and effective against free radical to reduce skin damage (Pullar, Carr *et al.* 2017). Whereas folate is used as an antiaging constituent, it promotes regeneration process of skin cell by providing micronutrients. It improves viability of UV-damaged skin cells by modulation of DNA repair mechanism (Ammar, Ghorab *et al.* 2016). In studies, it was shown that the high levels of folates and antioxidants can decrease the depth and severity of skin wrinkles by 60% (Fam, Holt *et al.*

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2020). 24-hr ABPM data was collected in four 100 independent randomised clinical trials testing the effects of beetroot juice supplementation. It was concluded that the vascular responsiveness to inorganic nitrate may be modified by mechanisms of vascular ageing influencing the reducing capacity to convert inorganic nitrate into nitrite and tissue-specific responses to dietary nitrate supplementation (Campa and Baron 2018).

Selenium and glycolic acid present in *Saccharam Officinarum* provide excellent protection free radicals. Selenoprotein, contains selenium, has antioxidant effects and are involved in regulating antioxidant activities, reduce DNA damage and prolonged telomere length and thereby plays roles in fighting aging and preventing aging-related diseases (Cai, Zhang *et al.* 2019). Glycolic acid is used to treat a number of skin conditions in which it acts as an exfoliating agent. It hydrates dry skin and reduce dark pigmentation of the skin. It also stimulates skin to make hyaluronic acid in the deeper layers, that hydrates skin to a dewy and youthful look and feel (Roberts 2004).

Punica granatum, is an effective antioxidant. *Punica granatum* extract protects human fibroblasts, in vitro, from UV-induced cell death; likely due to the decreased activation of NF-κB, downregulation of proapoptotic caspace-3, and increased DNA repair (Pacheco-Palencia, Noratto *et al.* 2008). The topical application of a microemulsion of *Punica granatum* extract using a polysorbate surfactant (Tween 80®) in a 12-week split-face comparison with 11 subjects, demonstrated decreased melanin (due to tyrosinase inhibition) and decreased erythema compared to the vehicle control (Parveen, Akhtar *et al.* 2014, Mo, Kaewnopparat *et al.* 2015).

Keeping in view the medicinal, especially anti-aging effects of *Beta Vulgaris*, *Punica granatum* seeds *and Saccharam Officinarum*, the objective of the present study was to formulate a confectionary product in the form of toffee which is desirable for different groups of people with its anti-aging potential. Newly established toffee is not only capable of producing anti-aging effects, but it is also ready to compete in market due to its taste and cheap cost. The clinical trials and market research may further be carried out expending the area of study.

Methodology

Ingredients and Glassware

Analytical grade chemicals were used for analysis. All glassware was pre-rinsed with 10% HCl followed by deionized water. The ingredients of toffee e.i. jaggery (local), Vanilla essence (Synarome), Strawberry essence (Fermenich) and food grade CMC (Daraz.pk) were purchased from super market located in Karachi.

Collection of botanical Ingredients

Beta Vulgaris was purchased from an A grade fruit and vegetable store, whereas dried *Punica granatum* seeds from an herbal store located at Nazimabad, Karachi. Botanical identification of the plants was done by the Botany Department of the University.

Preparation of the Ingredients

The dirt and other possible impurities of *Beta Vulgaris* were removed by washing the sample with distilled de-ionized water. The root was grinded and was immediately taken for further processing.

Preparation of toffee

Five samples of toffee were prepared with varying composition of *Beta Vulgaris and* jaggery in amount varies from 80/120, 90/110, 100/100, 110/90 and 120/80 gm/gm and constant amount of other ingredients. Previously described processing technique of with some modification was employed to prepare the toffee (Dobhal and Awasthi 2019). *Saccharam Officinarum* was heated on stainless steel pan until a thin paste is formed. Then *Beta Vulgaris* (washed and grinded) and heated at 60-70^{o}C with constant stirring for 18-20min. Then powdered seeds of *Punica granatum* were added and heated with constant stirring for 2-3min. Thereafter 2, 3 drops of lemon along with vanilla and strawberry flavoring essence were added with constant stirring. After mixing of all ingredients CMC was added to the mixture with constant stirring. The heated mass was spread on 1.5 to 2.5 cm stainless steel cubes molds (smeared with fat). The heated mass was cooled for three hours.

Organoleptic Evaluation

Standard 9-point hedonic scale procedure was used to carry out the sensory (organoleptic) evaluation of the finally produced toffees (Amerine, Pangborn *et al.* 1965). The mean score of each quality parameter such as overall acceptability, colour, texture, flavor and taste was recorded which was analysed by 20 trained/and semi trained panelist.

Packaging Material and Storage

The metallic coated polythene wrapper was used to wrap the final product, 50-micron Polyethylene bags were used to pack and were kept for 4 months.

Physical and Chemical Analysis

Physical tests of the final product such as moisture, water activity, pH, ash, colour and chemical analysis such as sucrose, reducing sugar and crude protein, crude fiber and reducing sugar were carried out according to the standard methods of AOAC (Chemists and Horwitz 1975). The texture was analyzed by texture analyzer, and the calorific value was estimated by Bomb Calorimeter.

Microbial Analysis

Standard Plate Count (SPC) method was used to record microbial counts. Petri dishes with Potato Dextrose Agar (PDA) as the cultivation medium were kept for incubation at $37 \pm 5°$ C for 2 days. The colonies were counted by colony counter.

Result and Discussion

Table 1: Standardization of Toffee Recipe

Parameters	**Control**	**Sample1**	**Sample2**	**Sample3**	**Sample4**	**Sample5**
***Saccharam Officinarum* contents (gm)**	0	80	90	100	110	120
***Beta Vulgaris* contents (gm)**	0	120	110	100	90	80
Organoleptic acceptability score*	8.30	8.20	8.25	8.75	8.2	8.15
Remarks	Control	Not Selected	Not Selected	**Selected**	Not Selected	Not Selected

* Nine point hedonic scale:

Keeping other ingredients constant five samples of toffee were prepared with varying composition of *Beta Vulgaris and Saccharam Officinarum* in amount varies from 80/120, 90/110, 100/100, 110/90 and 120/80 gm/gm. The sensory analysts recommended the sample with 100gm each of the component (Table-1). As compared to the control sample, both had almost the same taste, texture and mouth feel. It can be revealed from the sensory results that the acceptability of selected toffee was found to be above average.

The sensory score of freshly prepared toffee (Sample-3) has been summarized in Table 2. The colour of final product was observed to be 8.3 and 8.6 for control and selected sample of toffee. This might be due to intense colour of *Beta Vulgaris* and *Punica granatum*. The score for texture for control and selected sample was 8.4 to 8.7. Flavour score for control and the selected sample was found to be 8.5 to 8.8. The taste scores for control and the selected sample was observed as 8.3 and 8.8. Both of the products control and the selected sample were found to be acceptable. The composition and better sensory scores of both of the products might have made both of the product acceptable.

Table 2: Sensory score of fresh toffees

Parameters	**Control**	**Sample3**
Colour	8.3±0.04	8.6±0.01
Texture	8.4±0.04	8.7±0.03
Flavour	8.5±0.02	8.8±0.02
Taste	8.3±0.04	8.8±0.03
Organoleptic acceptability score*	8.30±0.04	8.75±0.03

* Nine point hedonic scale:

The physical analysis of control and the selected sample have been recorded in Table 3. Water activity was recorded as 0.6 and 0.56A_w for control and selected sample respectively. The basic reason for migration of moisture in both of the products is the difference in water activity. Rapid migration of moisture is due to increase in water activity in both of the products. The results of pH analysis have provided an evident result that it is suitable according to the product. Hardness is one of the variables that are used to determine the texture profile of the food. The hardness in newton is found 4.35N and 4.39N for control and the selected sample of freshly prepared toffee respectively. The texture of toffee is happened to be affected by the additional moisture content, the interaction between protein and fat-soluble flavonoids. Moisture and fat content governs the texture i.e. runny, soft, chewy etc. The calorific values indicate that the selected sample of freshly prepared toffee contains only 4.3% of the calories needed by the senior citizens in a day.

Table 3: Physical Analysis of the Fresh Toffees

Parameters	**Controlled sample**	**Sample3**
Water Activity (A_w)	0.6	0.56
pH	5.3±0.02	5.2±0.02
Hardness (deformation N)	4.39±0.4	4.35±0.5
Calories (kcal/5g)	43.6±1.8	85.7±2.1

The results of chemical analysis of the fresh final product are summarized in Table 4. Food quality can be maintained by maintaining less moisture content in

the food commodities; that will reduce the deterioration of food by decreasing microbial growth. In the current study the moisture content is found to be lesser which ascertains the quality of good product. The ash content in the controlled sample is found to be less than the ash content in the selected sample. It is mainly because selected sample is obtained from a plant source which could be the reason of higher value in the later product. The ash content in both of the samples are found to be in acceptable limits, which make the product acceptable. Reducing sugars are derived by hydrolysis of disaccharides. In this study, galactose, fructose be produced during thermal hydrolysis. The fat content is found to be less than the detection limit. Plate count value was found to be less than the detection limit. Confections are generally resistant to bacterial growth, but pathogens (if present) survival in the product for a long time is possible (Vanderzant and Splittstroesser 2001). The constituents of the product produced in the current study are natural, no artificial colour is added to prepare the toffee.

Antioxidant activity as IC50 was observed to be higher in 128.3ppm in control sample than 92.23ppm in freshly prepared anti-aging toffee, vitamin A present in *Beta Vulgaris* reverses age-related skin thinning stimulate, renewal of skin collagen and reversal of uneven skin pigmentation and vitamin C helps to reverse uneven skin pigment rebuild skin collagen and effective against free radical to reduce skin damage; hence affect the amount of antioxidant IC50. It has been reported in the literature that the total phenolic content is an indicator to determine the hydrophilic activity of antioxidants. The more the phenols present in the product, the lower IC50 antioxidant activity will be (Priecina and Karlina 2013). The lower IC50 values show better antioxidant activity of the toffee in the current study.

Moisture content of control sample was found lesser (4.8%) than the selected sample3 (5.6%). Due to difference in water activity migration of moisture in both of the products is observed. Higher the water activity; more rapid the migration of moisture can be observed.

Table 4: Chemical Analysis of the Fresh Toffees

Parameters	**Controlled sample**	**Sample3**
Moisture Content(%)	11.53±0.05	11.48±0.06
Ash(%)	0.77±0.02	0.80±0.01
Reducing Sugar%	13±0.25	12.6±0.62
Fat Content%	<Detection Limit	<Detection Limit
Crude Protein%	0.4 ± 0.02	0.60 ± 0.03
Crude Fiber%	0.4 ± 0.02	1.9 ± 0.03
Antioxidants IC50 (ppm)	128.3 ± 1.0	92.23 ± 0.9
Microbial Analysis		
Standard Plate Count	< the Detection Limit	< the Detection Limit

Storage treatment

The effectiveness of packaging material for the newly established anti-aging toffee was investigated. No change in flavour and texture were observed for the samples packed with aluminum laminated foil (ALU) whereas the samples packed with butter paper has shown lost in texture but aroma and flavor were retained on storing in cool and dry conditions, two places were elected for

storage i.e. cool and place and moist and wet place. Best results were found in laminated aluminum foil (ALU) packaging stored at cool and dry place. Aluminum provides a very good barrier to protect food against light and air and preserves food quality. Aluminum have 100% water and gas barrier. More than this, it's corrosion resistant to substances in the pH range from 4 to 9 (Renumarn and Choosuk 2020).

Table 5: Microbiological Quality Assessment of selected sample of toffee

S.No	Test Parameters	0 days	30 days	60 days	90 days	120 days
1.	Total aerobic bacteria, cfu/g	0.9×10^2	3.2×10^3	6.5×10^3	1.3×10^4	2.9×10^4
2.	Total Coliform, MPN/g	<0.3	<0.3	<0.3	<0.3	<0.3
3.	E. coli, MPN/g	<0.3	<0.3	<0.3	<0.3	<0.3
4.	Total yeast and molds, cfu/g	<10	<10	<10	2.6×10^2	10×10^2

<10 indicates absence of test organism in 1g of sample; MPN<0.3 indicates absence of test organism in 1g of sample

To ensure the best shelf life of around 4 months, toffee was stored at room temperature and less humid environment. In this study the microbial load of the presently developed toffee was assessed up to 4 months. According to Food Standards, the total aerobic plate count, total yeast, and mold count were within the acceptable limit, whereas no Coliform or *E. coli* was found up to 4 months. After 4 months, the hygienic indicator organisms were gradually increased and the product quality became deteriorating (Table 5).

The cost of newly developed toffee was calculated as per existing prices at the time of the study. It has been observed that the newly developed soup also has the market competent price therefore the product is likely to gain the attraction of consumer and improves the market trends.

Conclusion

In the present study a new nutraceutical (anti-aging) toffee was produced by incorporating of *Beta Vulgaris*, *Punica granatum* and *Saccharam Officinarum*. Among the various combination, the toffee containing 100g/100g *Beta Vulgaris* and *Saccharam Officinarum* was found better than other combinations in respect to organoleptic properties and nutritional quality. The moisture, ash, protein, fiber, reducing sugar, water activity, deformation and energy content of newly developed anti-aging toffee were found to be 11.48±0.06%, 0.80±0.01%, 0.60 ± 0.03%, 1.9 ± 0.03%, 12.6±0.62%, 0.56(A_w), 4.35±0.5N and 85.7±2.1kcal/100 g, respectively. The toffees prepared were wrapped in metallic coated polythene wrapper, packed in 50-micron polythene bags and stored for 4 months. The total yeast, aerobic plate count and mold count were found to be the acceptable according to food standards. After 4 months, the microbial counts were increased and deterioration in the product quality was observed. The calorific values browte that the newly developed anti-aging toffee contains only 4.3% of the daily calories needs of a senior citizen. The newly established toffee imparts anti-aging impact on the aged people due to medicinal importance of the ingredients. There are several confectionery products sold in the market which are utilized by the

elderly people and few of them have medicinal properties, but no toffee with anti-aging enhancing properties has been reported in the literature earlier. Therefore, the introduction of this first ever anti-aging toffee in the market will improve the health of the elderly people as well as the product cycle of the industry. It also has the market competent price therefor the product is likely to gain the attraction of consumer and improves the market trends.

Reference

Amerine, M, *et al.* (1965). "Principles of sensory evaluation of food Academic Press." New York/London.

Ammar, HO, *et al.* (2016). "Folic acid loaded lipid nanocarriers with promoted skin antiaging and antioxidant efficacy." Journal of drug delivery science and technology **31**: 72-82.

Cai, Z, *et al.* (2019). "Selenium, aging and aging-related diseases." Aging clinical and experimental research **31**(8): 1035-1047.

Campa, M and Baron, E (2018). "Anti-aging effects of select botanicals: scientific evidence and current trends." Cosmetics **5**(3): 54.

Chemists, AOOA and Horwitz, W (1975). Official methods of analysis, Association of Official Analytical Chemists Washington, DC.

Dobhal, A and Awasthi, P (2019). "Formulation of beetroot candy using simple processing techniques and quality evaluation." Journal of Pharmacognosy and Phytochemistry **8**(4): 913-916.

Fam, VW, *et al.* (2020). "Prospective Evaluation of Mango Fruit Intake on Facial Wrinkles and Erythema in Postmenopausal Women: A Randomized Clinical Pilot Study." Nutrients **12**(11): 3381.

Mo, J, *et al.* (2015). "Physicochemical properties, in vitro release and skin permeation studies of a topical formulation of standardized pomegranate rind extract." Pakistan journal of pharmaceutical sciences **28**(1).

Ninfali, P and Angelino, D (2013). "Nutritional and functional potential of Beta vulgaris cicla and rubra." Fitoterapia **89**: 188-199.

Pacheco-Palencia, LA, *et al.* (2008). "Protective effects of standardized pomegranate (Punica granatum L.) polyphenolic extract in ultraviolet-irradiated human skin fibroblasts." Journal of agricultural and food chemistry **56**(18): 8434-8441.

Parveen, R, *et al.* (2014). "Topical microemulsion containing Punica granatum extract: its control over skin erythema and melanin in healthy Asian subjects." Advances in Dermatology and Allergology/Postępy Dermatologii i Alergologii **31**(6): 351.

Priecina, L and Karlina, D (2013). Total Polyphenol, Flavonoid Content And Antiradical Activity Of Celery, Dill, Parsley, Onion And Garlic Dried Conventive And Microwave-Vacuum Dryers. International Conference on Nutrition and Food Sciences.

Pullar, JM, *et al.* (2017). "The roles of vitamin C in skin health." Nutrients **9**(8): 866.

Renumarn, P and Choosuk, N (2020). Influence of Packaging and Storage Conditions on the Quality and Shelf-life of Chewy Santol (Kraton-Yee) Candies. E3S Web of Conferences, EDP Sciences.

Roberts, WE (2004). "Chemical peeling in ethnic/dark skin." Dermatologic therapy **17**(2): 196-205.

Vanderzant, C and Splittstroesser, D (2001). "Compendium of Methods for the Microbiological Examination of Foods, (American Public Health Association, Washington, DC, USA)."