Agricultural Research & Technology
Open Access Journal
ISSN: 2471-6774

Juniper Publishers
key to the Researchers

Research Article
Volume 8 Issue 2 - July 2017
DOI: 10.19080/ARTOAJ.2017.08.555733

Agri Res & Tech: Open Access J

Extraction and Utilization of *Manihot esculenta crantz* and *Trapa natans* Starch as a Stabilizer in Soy Milk Based Ice Cream Preparation

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Submission: June 28, 2017; **Published:** July 17, 2017

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Abstract

Stabilizers are important ingredient in ice cream manufacturing. It improves mix viscosity, air incorporation, air cell distribution, texture, melting properties and minimize quality defect. Soy milk used for milk replacer due to its health benefits such as dietary fiber, protein, isoflavones, vitamin C, carotenoids, omega-3-fatty acid and oligosaccharides. Starches were extracted from *Trapa natans* and *manihot esculenta crantz* analyzed for different quality parameters for pH, water binding capacity, swelling power and solubility, moisture, protein and viscosity. The starch extracted from *Trapa natans* showed better results against all the quality parameters as compare to *manihot esculenta crantz* starch. Soy milk based ice cream was prepared using different percentages of (0.25, 0.5 and 0.75%) *Trapa natans* and *manihot esculenta crantz* starches as stabilizer. Cremodan stabilizer was used as reference standard. Ice cream was characterized for physicochemical and sensory attributes. Ice cream sample prepared with 0.75% of *Trapa natans* starch showed significant ($P<0.05$) results in term of high overrun, meltdown, standup time and viscosity. The development of soy milk based ice cream with novel starches from natural sources provides extra health benefits and reduce the cost of production.

Keywords: *Manihot esculenta crantz; Trapa natans;* Starch; Stabilizer; Soy milk, Ice cream preparation

Abbreviations: SNF: Solid Not Fat; cP: Centipoise; CRD: Completely Randomized Design; SPSS: Statistical Package for the Social Sciences

Introduction

A novel trend in functional foods has been pointed out as the production of non-dairy food products [1]. Functional ingredients such as, prebiotic, probiotic, soy and their derivatives grow 5% per year in the market of food products worldwide [2]. Soy based products have potential for alternative of cow milk. Soybeans are good source of carbohydrates and proteins (high quality) but free from cholesterol and lactose. It is a potential milk substitute source for milk allergy patients (lactose intolerance) and vegetarians [3]. Soybean proteins affect the cholesterol metabolism and fecal excretion of bile acids [4]. Soy milk have different health benefits such as dietary fiber, protein, isoflavones, vitamin C, carotenoids, omega-3-fatty acid and oligosaccharides. Soybean composes of 15-16% dietary fiber, 39-40% protein, 14-15% saccharides, 19-20% oil contents, and 10-11% others miscellaneous nutrients [5].

Stabilizers are important ingredients of ice cream manufacturing. The amount of stabilizer used depends upon desired quality of the end product. The stabilizers during dispersion hold the flavoring compound provide good aroma and help to hinder air bubbles from collapsing [6]. The main functions of stabilizer are to increase the mix viscosity, prevented separation of proteins, whipping properties improvement, texture development, retarded ice crystal growth, increase the melting resistance and regulate the sensory properties [7].

Trapa natans is an important native crop in Asia and many other parts of world. Due to its unique taste, it is a significant product in food industry [8]. *Trapa natans* is a good source of starch present large prospective for commercial use. *Trapa natans* for domestic and industrial use, is great source of starch due to its more carbohydrate contents. Because of their characteristics

like: solubility, gelatinization, freeze thaw stability, swelling properties starches are famous to impart functionality in range of dairy products [9]. Starches are used as coagulating agents (pie fillings, cream soups and sauces), gel-forming agents (gum confections), moisture retainers (cake toppings), colloidal stabilizers (salad-dressings), binders (cones, wafers), coating agents (candies, nutmeats) and varnishing. Functions of starch depend upon its physicochemical properties in various foods [10]. Uses of starches in products are mainly directed due its pasting, solubility, gelatinization, swelling, digestibility and color [11].

Manihot esculenta crantz with tuberous roots is a perennial woody shrub [12]. In Asia, America (Latin) and Africa *Manihot esculenta crantz* after maize and rice cultivated as the third most important source of calories. In 2012, its production in all over the world was evaluated as 262,585,741 tons [13]. Availability of *Manihot esculenta crantz* is around the year due the planting and harvesting flexibility of crop. Nutritionally, its roots assumed a better source of dietary fiber, riboflavin, magnesium, nicotinic acid, citrate and thiamin, but not good source of protein [14]. Excluding consumption, its roots is mostly used for different industrial purpose such as ethanol and biofuel production [15]. Starch form *Manihot esculenta crantz* roots are the vital component and obtained up to 80% of dried weight [16]. Application in food industries as thickeners (soups, baby foods, sauces, and gravies), fillers, binders (sausages and processed meats) and as a stabilizers (ice cream) [17].

Worldwide population is regularly increase and malnutrition in developing countries is occurred due to insufficient supply of protein. Protein from animal source is expensive in developing countries, fulfil the demands of protein, research effort to finding alternative sources of protein from legume seeds [18]. Due to significance of soy milk and carbohydrate the present study was planned to add the manihot esculenta crantzand *Trapa natans* starches in different concentrations in the locally prepared soy milk for the preparation of ice cream.

Material and Methods

Procurement of raw material

Soya beans and *Trapa natans* was purchased from the local market for soy milk preparation and starch extraction respectively. *Manihot esculenta crantz* roots was purchased from Zamzam Enterprises, Global Exporters of fresh products are based in Karachi, Pakistan. Other ingredients such as sugar, egg yolk, stabilizer (*Trapa natans* and *Manihot esculenta crantz* starch) artificial flavor and food grade color (FD&C yellow, 5) were purchased from the local market. For physicochemical analysis of soy milk, ice cream and starches all chemical and reagents were acquired from Sigma Aldrich (USA), Merck (Germany) and Oxoid (UK).

Preparation of soy milk

Soybeans (200g) were washed in de-ionized water. The cleaned beans were soaked in 1L of water for 13-14 h at 4-5 °C. Then swollen soybeans after separation were blend at low speed in 1 liter of boiling water for 5-6min. The obtained slurry was heated for 10min at 80 °C and then filtration was done through four layers of cheese cloth. The soy milk filtrate (1650g) was collected and cooled at room temperature and then kept at 4 °C [19] (Figure 1).

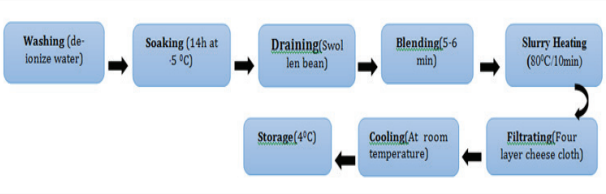

Figure 1: Flow diagram of the preparation of soy milk.

Analysis of soy milk

The pH of soy milk was measured by using the electronic digital pH meter (Inolab WTW Series 720) [20]. Fat, total nitrogen, ash, acidity and total solid contents were estimated by the method described by AOAC [20]. The solid not fat was calculated according to the method described Kirk & Sawyer [21].

Starch extraction

Starch from *Trapa natans* and *Manihot esculenta crantz* were extracted by using the method described by Tulyathan et al. [22] and Benesi et al. [23] respectively.

Analysis of starches

The pH of both starches was measured by using the electronic digital pH meter. Buffer solution of pH 4 and 7 were used for calibration of pH meter [24]. Fat, moisture, ash and total nitrogen content were evaluated according to method AOAC [20]. Water binding capacities of dried starches were estimated by the method described by Garg & Jana [25]. The viscosity was measured by using Viscometer by the method of Mweta [26]. Swelling power and solubility were calculated according to the method of Garg & Jana [25].

Preparation of ice cream

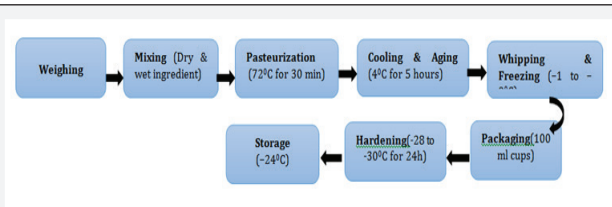

Figure 2: Flow diagram of the preparation of ice cream.

The dry ingredients sugar and stabilizers were weighed and mixed with liquid ingredients by constant stirring. Starches were used in different concentrations (0.25, 0.5 and 0.75%) for ice cream preparation as shown in Table 1. The obtained mixture was pasteurized at 72 °C (30min) and then by using electric homogenizer homogenization was done. After that, ageing was done at 4 °C (5h). The mixture was further subjected -1 to

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-9 °C along with whipping. For physicochemical and sensory evaluation ice cream was filled in 100ml disposable cup [27] (Figure 2 & Table 1).

Table 1: Different concentrations of Trapa natans and Manihot esculenta crantz for the preparation of soy milk based ice cream.

Treatments	*Trapa natans* Starch	Manihot Esculenta Crantz Starch	Cremodon (%)	Total (%)
T0	0	0	0.5	0.50%
T1	0.25	0	0	0.25%
T2	0	0.25	0	0.25%
T3	0.5	0	0	0.50%
T4	0	0.5	0	0.50%
T5	0.75	0	0	0.75%
T6	0	0.75	0	0.75%

Physicochemical analysis ice cream

Ice cream pH was measured with the help of pH meter according to the method of AOAC [24]. Overrun of prepared samples were estimated according to the Varnam & Sutherland [28]. Melt-ability of all prepared ice cream samples were estimated by the method described by Olsonet al. [29]. 100gm of ice cream was placed on mesh which fitted on a beaker. The melted ice cream weight after every 15min interval up to 75min was recorded at 26±3 °C . Mix viscosity (cP) was calculated by using Brookfield viscometer spindle No. 4 at 100rpm just before and after aging at 25 °C and 5 °C [30]. Standup time of ice cream mix was measured by method of Bhandari [31].

Sensory evaluation

After storage at -22 °C for 24 hours, all samples were judge by 5 staff members of Institute of Home and Food Sciences, Government College University Faisalabad according to the method described by Larmond [32]. Expert panel evaluates the impact of *Trapa natans* and *Manihot esculenta crantz* as stabilizers on taste, flavor, texture and overall acceptability of ice cream.

Statistical analysis

Data statistically analyzed for the effects of the factors on pH, viscosity, standup time, over-run, melt-ability and sensory evaluation was done by CRD using SPSS software to estimate the significance level [33]. The factors were: type of stabilizers (*Trapa natans* and *Manihot esculenta crantz* starch) and concentration (0.25, 0.5 and 0.75%).

Results and Discussion

Analysis of soy milk

Soy milk was analyzed for different physicochemical parameters. Mean value of these perimeters are following, such as pH (6.72-6.75), acidity (0.28.0.30%), ash (0.7-0.73%), protein (3.3-3.32%), SNF (10-10.2%) and total Solids (12.7-12.74%). Obtained results from present study are in line with the findings of Jooyandeh [34].

Analysis of starches

Trapa natans and *Manihot esculenta crantz* were subjected for different physicochemical analysis and all were performed in triplicate. Mean values for *Trapa natans* starch of all performed physicochemical parameters are following; pH (5.58±0.35), fat (0.25±0.01), water holding capacity (81.73±1.50), swelling power (3.0±0.2), solubility (2.3±0.7), moisture (9.5±0.5), protein (0.5±0.03) and viscosity (3394±55cP). Mean values of physicochemical analysis for *Manihot esculenta crantz* starch are following; pH (5.36±0.25), fat (0.25±0.01), water holding capacity (78±1.05), swelling power (2.5±0.3), solubility (1.8±0.2), moisture (8.3±0.5), protein (0.1±0.02) and viscosity (2870±35cP). The pH, water holding capacity, swelling power, solubility, moisture, crude protein and viscosity of *Trapa natans* starch were better than *Manihot esculenta crantz* except fat content (Table 2). The results regarding to physicochemical characteristics of starches are in line with the finding of Oladebeye et al. [35] and Gomand et al. [36] (Table 2).

Table 2: Physicochemical analysis of *Trapa natans* and *Manihot esculenta crantz* starches.

Parameter	*Trapa natans* starch	Manihot esculenta crantz *starch*
pH	5.58±0.35	5.36±0.25
Fat (%)	0.25±0.01	0.30±0.02
Water binding capacity (%)	81.73±1.50	78±1.05
Swelling power (%)	3.0±0.2	2.5±0.3
Solubility (%)	2.3±0.7	1.8±0.2
Moisture (%)	9.5±0.5	8.3±0.5
Crude protein (%)	0.5±0.03	0.1±0.02
Viscosity (cp)	3394±55	2870±35

Table 3: Comparison of mean values for physicochemical characteristics influenced by treatments.

Treatment*	pH			Viscosity (cP)		
	Fat (%)	Before Aging	After Aging	Before Aging	After Aging	Overrun %
To	2.51±.02	7.29±0.05	7.36±0.10	2950±20	4250±18	53.54±0.26
T1	2.45±.01	7.19±0.07	7.25±0.11	2360±21	3460±17	44.76±0.64
T2	2.47±.03	7.15±0.08	7.22±0.09	1950±18	2750±14	39.48±0.60
T3	2.48±.03	7.25±0.04	7.31±0.07	2646±16	3846±20	47.85±0.58
T4	2.49±.04	7.20±0.06	7.26±0.11	2126±15	3026±21	41.76±0.70

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T5	2.51±.02	7.30±0.03	7.37±0.08	3053±16	4453±15	54.68±0.65
T6	2.52±.05	7.26±0.09	7.30±0.06	2443±15	3543±22	44.20±0.52

Analysis of soy milk base ice cream

It was clear from all statistical outcomes fat content significantly (P<0.05) varies among all the treatments. Highest value for fat content (2.52±.02) was obtained for T6 having 0.75% concentration of *Manihot esculenta crantz* (Table 3). The fat content varies among all the treatment due to the fat content difference present in both starches and among all the treatments concentration of starches were different.

Statistical results indicate that pH of all ice cream samples significantly (p<0.05) differ among all treatments. On aging, pH value of all treatments were increased, this trend is accordance with Akesowan [37]; Rezaei et al. [38]. The highest mean value of pH before the aging (7.30±0.03) and (7.37±0.08) after the aging were recorded in T5 while other have low pH. In dairy products, due to compositional and biochemical changes, pH has direct influence on the flavor perception during aging [39]. Results shows that the ice cream containing 0.75% concentration of *Trapa natans* starch had the least decreased in pH and due to less production of acidity.

Statistically, the effect of different concentrations of stabilizers on the viscosity of soy milk ice cream found significant (P<0.05) among all the treatments. The interaction of stabilizers was found linear, with increasing the concentration of starch viscosity also increase. The highest value of viscosity before aging (3053±16) and (4453±15) after aging was observed for *Trapa natans* starch at concentration of 0.75% as compare to the controlled. The factors affecting the viscosity during this study, temperature, fat globule size, protein hydration, type and concentration of stabilizers. Flavor and mouth feel of ice cream mix was provided by viscosity [40]. Viscosity is also increase if fat and protein content increase, but these components in all samples remain same. So, viscosity was increase only due to different type and concentration of stabilizers [41]. Rheological properties of ice cream affected due to high water holding capacity of starches [42,43].

Overrun among all treatments also significantly (P<0.05) effected, because stabilizer type and concentration had a significant effect. The highest value (54.68±0.65) was observed for *Trapa natans* starch at 0.75% concentration shown in Table 3. Ice crystals and air cells become smaller if overrun decrease [44]. Ice cream volume loss due to collapse of weakened film reported by Potter and Hotchkiss [45]. Air loss from ice cream mix the shrinkage was reported by Rothwell [46]. Air incorporation is important due to overrun property, product quality and profits. The less and more air incorporation produce soggy and fluffy ice cream respectively [47] (Table 3).

After melting first drop of ice cream fell was noted for each sample. Addition of both starches at different concentrations showed significant (P<0.05) effect on the stand up time of soy milk ice cream (Table 4). At 0.75% concentration of *Trapa natans* starch, highest mean value of standup time before and after aging were observed, 12.10±0.12 and 18.10±0.14 respectively. While the lowest values were observed in ice cream sample T4 before and after aging shown in Table 3. Investigation shows that significant variation in standup time, by increasing the quantity of starches and due to their binding ability. Normal standup time for ice cream is 13 minutes at 20 °C [48].

Table 4: Comparison of mean values for physicochemical characteristics influenced by treatments.

Treatments*	Standup Time (min.)		Melt ability % Time (min.)				
	Before aging	After aging	15	30	45	60	75
To	11.20±0.10	17.20±0.17	1.32	5.28	19.93	40.93	62.52
T1	9.45±0.14	14.35±0.18	1	4	24.2	45.2	66.65
T2	7.21±0.11	10.21±0.10	2.02	8.06	30.2	51.2	70.34
T3	10.2±0.15	15.18±0.11	0.66	2.64	21.2	41.2	62.89
T4	8.01±0.12	12.10±0.13	1.56	6.2	27.56	47.2	67.75
T5	12.10±0.12	18.10±0.14	0.34	1.38	18.96	39.23	60.23
T6	9.05±0.13	13.05±0.10	1.2	4.8	26.5	45.45	65.2

*See Table 1: (LSD at 0.05 value for melting resistance; Treatments=0.556; Time=0.5625; Interactions=1.1797).

Melt-ability shows that the melting resistance of ice cream samples. Melt-ability was decrease, due to the air incorporation and quality of end product increased. Statistically, it was observed that all melt-ability significant (P<0.05) varies among all treatments according to time (0, 15, 30, 45, 60 and 75min.). Overall the highest (70.34) and lowest (60.23) melt-ability after 75min were observed for T2 and T5 (Table 4). The factors effecting the melt-ability of ice cream, composition, additives and many other, air incorporated, ice crystals, fat globules, type and nature of stabilizers [49]. The ice cream sample having highest overrun began to melt slowly and vice versa due to binding ability of starches. Low freezing point and environmental conditions are primary reason of rapid melting [48]. Melt-ability of the ice cream is reduce, by the controlling heat transfer, outside temperature and homogenization [50](Table 4).

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Sensory evaluation of soy milk base ice cream

Table 5: Comparison of mean values for sensory characteristics influenced by treatments.

Treatment	T0	T1	T2	T3	T4	T5	T6
Flavor	7.4±0.54	6±0.83	5.4±0.54	6.8±0.70	5.8±0.89	7.8±0.83	6.2±0.81
Taste	7.6±0.54	6.2±0.70	5.5±0.50	6.9±0.81	5.9±0.53	7.9±0.70	6.4±0.85
Appearance	7.7±0.83	6.2±0.83	5.6±0.52	7.2±0.83	6.4±0.54	8.0±0.70	6.8±0.80
Body/Texture	7.6±0.54	5.8±0.80	5.4±0.50	6.7±0.70	5.8±0.89	7.8±0.83	6.4±0.80
Overall acceptability	7.8±0.83	6.0±0.70	5.8±0.80	7.0±0.83	6.2±0.85	8.0±0.70	6.6±0.70

Sensory properties of soy milk ice cream as affected by different type and concentration of stabilizers (Table 5). Samples were evaluated for appearance, taste, flavor, body and overall acceptability, by the panel of 5 judges. All the sensory parameters were significantly affected by the concentration of *Trapa natans* and *Manihot esculenta crantz* as compare to control. The ice cream sample get highest awarded by judges panel containing 0.75% *Trapa natans* starch followed by the ice cream containing 0.5% *Trapa natans* starch. While ice cream containing *Manihot esculenta crantz* as a stabilizers got the lowest scores. Difference in all sensory parameters was observed due the difference of starch color and taste because the *Manihot esculenta crantz* is sour or fermented taste and dark in color on drying but *Trapa natans* is sweet in taste and white in color (Table 5).

Conclusion

Locally prepared soy milk was used successfully to prepare soy milk based ice cream. Main purpose of this study to prepared soy milk based ice cream for consumers due to their different health benefits anti-allergenic, anti cholesterolemic, anti atherogenic and hypolipidemic properties. Recommended stabilizer for ice cream could be *Trapa natans* starch at 0.75% for best viscosity, over-run, meltdown, standup time and sensory parameters. It is concluded that ice cream made with locally available *Trapa natans* starch as stabilizer showed the comparable results from commercially used imported stabilizer. Therefore, by using locally available stabilizers, the production cost can be minimized and foreign exchange can be saved. The ethical concerns have resulted in a global interest for Halal and natural stabilizer. Moreover, soy milk based ice cream also has potential for commercialization as a frozen dessert.

Acknowledgments

It certify that the author do not have any affiliation for any financial interest with in any organization.

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DOI: 10.19080/ARTOAJ.2017.08.555733

How to cite this article: Muhammad F M, Nazir A. Extraction and Utilization of *Manihot esculenta crantz* and *Trapa natans* Starch as a Stabilizer in Soy Milk Based Ice Cream Preparation. Agri Res & Tech: Open Access J. 2017; 8(2): 555733. DOI: 10.19080/ARTOAJ.2017.08.555733.