Stabilization of Ice-cream by incorporating α-Amylase Modified Taro (Colocasia esculenta) Starch

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Abstract ---**In present study, an attempt extracts the starch from taro (Colocasiaesculenta) by standerd wet milling extraction process. Extracted starch was then enzymatically modified by using α-amylase (Porcelain pancreatic amylase). The sample prepared by enzyme treatment was designated as ETMS and extracted starch was used as control sample (ES). Starch after treatment was dried and packed in polyethylene bag. ES and EMTS samples were analyzed for; amylose content (%), water absorption capacity (g/g), swelling power (g/g), solubility (g/g), enzyme digestibility (ED %) and dispersibility (%). functional parameters of EMTS were compared with ES. Result showed that the functional properties for EMTS were amylose content (15.39%), swelling power (17.43g/g), solubility (0.098g/g), water absorption capacity (1.53g/g), enzyme digestibility (62.70%) and dispersibility (75%), while for ES amylose content (17.83%), swelling power (16.02g/g), solubility (0.098g/g), water absorption capacity (1.64g/g), enzyme digestibility (59.32%) and dispersibility (83%). Data reveal that functional properties incsluding (swelling power and enzyme digestibility) were improved in EMTS. It was reported that amylose content, dispersibility and water absorption capacity was slightly reduced on enzyme treatment owing to denaturation amylose. Solubility was remains unchanged in both starches. Ice-cream is prepared by incorporating EMTS at 0.3%, 0.5% and 1 %. Sample ICEM 0.5 reported as best by sensory evaluation. Further sample ICEM 0.5 analyzed for Viscosity (Cp), % overrun and foam stability (for 120 min duration) by taking ICGG (0.4% guar gum incorporated Ice-cream sample) as control sample. Results for ICEM 0.5 showed that the viscosity (110.21Cp) and overrun (65.67%) were higher than viscosity (92.72 Cp) and overrun (62.31%) of ICGG. Sample ICEM 0.5 was also more stable than ICGG. Study concludes that modified taro starch can be prepared by enzyme modification (α-amylase) and useful as stabilizer in ice-cream with effective foam stability, viscosity and overrun.**
Keywords: **Taro Starch, centrifugation, α-amylase, EMTS, swelling power, foam stability, viscosity, overrun.**

I.INTRODUCTION

Starch is a carbohydrate consisting of a large number of glucose units joined together by glycosidicbonds. This polysaccharide is produced by all green plants as an energy store.It is the most common carbohydrate in the human diet and is contained in copious amounts in such staple foods as potatoes, wheat, maize (corn), rice, and cassava. Depending on the plant, starch contains 20 to 25% amylose and 75 to 80% amylopectin[1].

A long stalked herbaceous plant with huge leaves, growing to the height of 30 to 150 cms. Rootstock is tuberous up to 10 cm in diameter. The leaves in groups of two or three are long petiolaed, ovate, 20 to 50 cm long, glaucous, with the insertion of the petiole, with broad and rounded basal lobes. Petioles are green or purplish, 0.2 to 1 m long. Spathe is variable in length, about 20 cm long. Spadix is cylindric, half as long as the spathe, green below and yellowish above.

Taro belongs to the genus Colocasia, within the sub-family Colocasioideae of the monocotyledonous family Araceae. Because of a long history of vegetative propagation, there is considerable confusion in the taxonomy of the genus Colocasia. Cultivated taro is classified as Colocasiaesculenta, but the species is considered to be polymorphic. There are at least two botanical varieties: i) Colocasiaesculenta (L.) Schott var. esculenta; ii) Colocasiaesculenta (L.) Schott var. antiquorum (Schott) Hubbard &Rehder which is synonymous with C. esculenta var. globulifera Engl. & Krause. C. esculenta var. esculenta is characterised by the possession of a large cylindrical central corm, and very few cormels. It is referred at agronomically as the dasheen type of taro. C. esculenta var. antiquorum, on the other hand, has a small globular central corm, with several relatively large cormels arising from the corm. This variety is referred to agronomically as the eddoe type of taro. Most of the taro grown in the Asia/Pacific region is of the dasheen [2].

Taro corm is an excellent source of carbohydrate, the majority being starch of which 17-28% is amylase, and the remainder is amylopectin [3]. Taro is especially useful to people allergic to cereals and can be consumed by children who are sensitive to milk, and as such taro flour is used in infant food formulae and canned baby foods. It contains greater amounts of vitamin B-complex than whole milk [4]. Proximate composition of the taro corm on a fresh weight basis include; Moisture 63-85%, Carbohydrate (mostly starch) 13-29%, Protein 1.4-3.0%, Fat 0.16-0.36%, Crude Fibre 0.60-1.18%, Ash 0.60-1.3%, Vitamin C 7-9 mg/100 g, Thiamine 0.18 mg/100 g, Riboflavin 0.04 mg/100 g, Niacin 0.9 mg/100g [5]. In Pacific Island countries such as Fiji and parts of Africa, taro is a staple food crop [6], [7].

Dairy food products which are whipped to add air bubbles into the products are known as a complicated colloid system. Ice cream is one such product which contains air bubbles or foams dispersing in the unfrozen phase. Air bubbles or foams contribute to a soft texture, light body of ice cream and retardation of ice cream melting [8]. The volume of air bubbles or foams in ice cream relates to the overrun which is normally reported in percentage. The higher the overrun the softer the ice cream, resulting in a higher profit. However, higher overrun with many small air bubbles may cause a fluffy texture which is less acceptable to consumers [9]. On the other hand, even a small amount of large foaming can cause ice cream collapse from Ostwald repining

[10]. To solve these problems and stabilize the air bubbles, quite many studies have been undertaken employing stabilizers to inhibit or limit the movement of air bubbles [11][12][13].

Stabilizers used in ice cream are normally polysaccharide gums, which serve to enhance viscosity in the ice cream mix and in the unfrozen phase of the ice cream following frozen process. They contribute to ice cream structure and alter textural characteristics [14]. The stabilizers mostly used in ice cream production are locust bean gum (LBG), guar gum, κ-carrgeenan and other commercial stabilizers e.g. Fulfil 400 which contains a mix of gums and emulsifier.

Using stabilizer replacers such as enzyme modified starch does not only lower the cost, but might also result in a better texture and lower melting point since they have been proven to inhibit freeze-thawing very well [15][16]. Such modified starches i.e. octenyl succinic anhydride starch (OSA), acetylated starch (AS) and hydroxypropylated starch (HPS), are thus widely used in frozen food products. Modification improve properties over the native form in storage stability, high swelling, high solubility, viscosity, low gelatinizaton, freeze-thaw stability and resistance to retrogradation[16][17].

Motive of the study is to develop the Enzyme modified starch with improved functional properties Viz. amylose content, water absorption capacity, swelling power, solubility, enzyme digestibility (ED) and dispersibility. Improvement of quality of ice cream i.e. viscosity, overrun and foam stability using enzyme modified taro starch by taking commercial guar gum as control sample.

II. MATERIALS AND METHODS

Fresh Taro tubers were purchased from local market of Jalgaon of Varity C. esculenta var. globuliferaEngl

2.1 Extraction of Starch from Tubers

Taro tubers was collected and cleaned by proper washing. After washing of tubers outer skin was removed by peeling. The tubers ware then sliced and choped and kept for drying in hot air oven at 55 ^{0}C. The dried tuber pieces were then crushed in mixer grinder to form the powder. This powder is further used for isolation of starch. Starch was isolated by standerd wet milling process

Taro starch was isolated according to the procedures used by Azhar Ahmed, Farukh Khan (2013) [18]. Taro powder (50 gm) was dispersed in a sodium metabisulphite solution (150 ml, 0.45% W/V) for 12 hours at refrigeration temperature. Now the slurry is being milled using a laboratory food blender for 5 min. The blended slurry was mixed with 450 ml NaCl solution (0.1 M) and 50 ml Toulene. The mixture was agitated for 1hr and then allowed to stand until the starch granules precipitate at the bottom. The protein in toluene and NaCl solution layers was siphoned off and discarded, and this was repeated until all of the proteins were removed as indicted by a clear toluene layer after the starch granules settled at the bottom. The starch layer was then washed with water several times and subsequently with absolute ethanol. The wet milled starch was then recovered by filtration through Whatman filter paper, rinsed with ethanol and air dried.

2.2 Enzyme modified Taro Starch Preparation (α-amylase treatment):

Enzyme modification of starch was achieved using the enzyme ∞-amylase (Porcelain pancreatic amylase). 40% starch slurry was prepared by adding 40gm of starch to 100ml of distilled water, and enzyme (100µl) was added. The samples were allowed to stand at room temperature for 6 h. The flasks were then kept in the incubator at 50^{0}C for 3h. After incubation, the supernatant was decanted and the starch was filtered and dried in the oven at 50^{0}C [19].

2.3 Characterization of Isolated starch and instant starch.

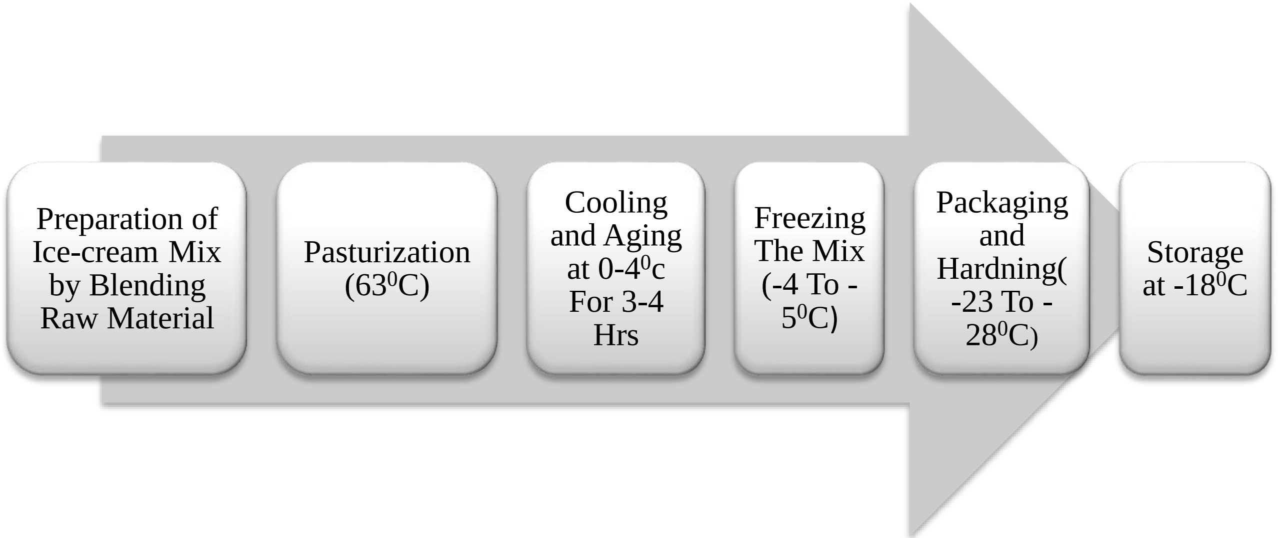
TABLE 1
METHODS FOR THE PHYSICOCHEMICAL ANALYSIS AND INSTRUMENTS

Physicochemical analysis	Method and instrument.
Moisture(%)	S. Ranganna1995, Hot air oven
Ash(%)	S. Ranganna1995, Muffle furnace
Fat(%)	S. Ranganna1995, solvent extraction method
Protein(%)	S. Ranganna1995, Protein analyzer (kjaldhal)
Crude fiber(%)	S. Ranganna1995, Crude fiber analyzer
Carbohydrates (%)	S. Ranganna1995[20], UV-Double Beam Spectrophotometer 2205
Amylose content (%)	Morrison and Laignelet (1983)[21], UV-VIS Spectrophotometer
Swelling power (g/g)	JiraratTattiyakul et al (2006)[22], laboratory centrifuge REMI-R4C
Solubility (g/g)	
WAC (g/g)	Medcalf and Gilles (1965)[23], laboratory centrifuge REMI-R4C
ED (%)	S. Ranganna, 1995, Incubator.
Dispersibility (%)	AACC 56-61A, 2000b[24]. measuring cylinder

2.4 Fourier transforms infrared spectroscopy (FTIR)

Fourier transform infrared spectra were recorded using a Jasco FTIR spectrometer. Starch Particles were collected using the KBr pellet method. A total of 32 scans were Obtained and the resolution was 4 cm-1. The wavelength region was between 4000 and 400 cm-1. All spectra were baseline corrected and normalized through setting the maximum transmittance to 100%[25].

2.5 Process of ice cream making



[26].

2.69-Points Hedonic Scale Sensory Evaluation.

The organoleptic characteristics of sweetened condensed milkwere evaluated. The panel member ware trained (Food Technologist) and untrained (Consumers). The panelists were asked to evaluate the sweetened condensed milkon the basis of approval of the Flavor, color, texture, appearance, mouth feel, after taste and overall acceptability on a 9-point hedonic scale. The value scales ranged from 9 (like, extremely) to 1 (dislike extremely) for each organoleptic attribute. Samples were served on white plastic dishes presented in random. For rinsing between samples, drinking water was available to the assessors [27].

2.7Characterization of Ice-cream

2.7.1Viscosity measurement

Viscosity measurements of solutions and mixes, before and after aging, were determined using Brookfield viscometer (D III Ultra; Brookfield Engineering Laboratory Inc., Middleboro, MA, USA) at 5°C with spindle no. 21 at 150 rpm. Readings were recorded after 5 sec of a measurement process. Viscosity measurements were performed in triplicate.[28]

2.7.2 Foam stability measurements

A food mixer(Moulinex BM9; Jebsen& Jessen, Bangkok, Thailand) with a 2.7l stationary bowl and rotating beaters was used for foam formation. 200l of ice cream mix were whipped at a turbo speed setting (planetary rpm of 220 and 5 cm diameter beaters rpm of 730) for 15 min. The whipping was performed at room temperature. Foam and mix were poured to a scaled cylinder to measure total volume of foam. Foam stability was carried out by plotting graph between total volume of foam and time of observation from 0 to 240 min[28].

2.7.3 Overrun measurement

Overrun measurements were begun immediately after the mixes were batch frozen in an ice cream batch freezer (Taylor 103-34; Taylor Rockton, IL, USA) [28]. The freezer was run under whipping and cooling conditions until the temperature of ice cream was lowered to the desired draw temperature (-5°C) and until the running process reached 15 min. Once the end point was reached the overrun in the ice cream was measured and calculated by comparing the weight of a known volume of ice cream to the weight of the same volume of unfrozen ice cream mix.

III RESULT AND DISCUSSION

TABLE II
RESULTS OF THE COMPOSITIONS OF TARO TUBERS

Parameter	Gram %
Moisture	72.5±0.73
Ash	0.94±0.01
Fat	0.26±0.01
Protein	2.45±0.08
Crude fiber	1.21±0.02
Carbohydrates	22.64±0.73

Table II showed chemical composition of Taro Tubers of verity C. esculenta var. globuliferaEngl Proximate compositions of physical parameters was in between the standard literature values results showed that the parameters were moisture 72.5 %, Ash 0.94 %, fat 0.26 % And protein 2.45 % but the crude fiber content of taro tuber was comparatively higher than that of the literature value (1.21%).

Figure 1: Standard FTIR Spectroscopy of extracted Taro Starch

Figure 1 showed the fingerprint region of the spectrum, three characteristic peaks appear between 929 and 1158/cm, which are attributed to C–O bond stretching. The peaks at 1086 and 1019/cm are characteristic of O–C stretching associated with the anhydroglucose ring. Peak near 850 cm−1 corresponded to the C–H deformations another characteristic peak occurs at 1644/cm, which may be related to the presence of tightly bound water in the starch. The peaks appearing at 3381–3800 /cm in the spectrum were due to hydrogen-bonded hydroxyl groups (O-H), which is attributable to the complex vibrational stretches associated with free, inter-, and intra-molecular bound hydroxyl groups, which make up the gross structure of starch. The sharp band at 2930/cm is characteristic of C–H stretching associated with the ring methane hydrogen atoms.

3.1 Results of the physicochemical parameters of extracted starch andEMTS.

TABLE III

PHYSICOCHEMICAL PARAMETERS OF EXTRACTED STARCH AND EMTS.

Parameters	Extracted Starch	EMTS
Amylose content (%)	17.83±0.01	15.39±0.01118
Swelling power (g/g)	16.02±0.01	17.43±0.01
Solubility (g/g)	0.098±0.00	0.098±0.00
WAC (g/g)	1.64±0.01	1.53±0.02
ED (%)	59.32±0.28	62.7±0.30
Dispersibility (%)	83±1.22	75±0.90

Results of the physicochemical analysis of extracted starch and EMTS are shown in Table III. As per the result obtained from the analysis. There was resulted in improvement of functional properties Viz. Swelling power (18.28 g/g) and enzyme digestibility (62.7%) which was higher than that of extracted starch (17.43g/g and 59.32%) While water absorption capacity and dispersibility of Extracted starch is quietly reduced it may be due to retrogradation on storage. Amylose content is decreased due to enzyme degradation of taro starch. There was no meaningful change in the solubility of starch.

3.2 Enzyme modified taro starch incorporated ice cream formulations

TABLE IV

ENZYME MODIFIED TARO STARCH INCORPORATED ICE CREAM FORMULATIONS

Sr.No.	Ingredients	ICGG	ICEM 0.3	ICEM 0.5	ICEM 1.0
1.	Milk fat	20	20	**20**	20
2.	Skim milk powder	11	11	**11**	11
3.	Sugar (sucrose)	17	17	**17**	17
4.	MS (Stabilizer)	0.4	0.3	**0.5**	1.0
5.	Emulsifier	0.25	0.25	**0.25**	0.25
6.	Water	65	65	**65**	65
7.	Vanilla essence	0.5	0.5	**0.5**	0.5
8.	Whole milk	15	15	**15**	15

ICGG (Control): Ice cream prepared by incorporating 0.4% Guar gum.

ICEM 0.3: Ice cream prepared by incorporating 0.3% Enzyme modified starch.

ICEM 0.5: Ice cream prepared by incorporating 0.5% Enzyme modified starch.

ICEM 1.0: Ice cream prepared by incorporating 1.0% Enzyme modified starch.

3.3Sensory evaluation of ice cream

TABLE V
SENSORY HEDONIC CHART OF DIFFERENT FORMULATIONS OF ICE CREAM

Sr. No.	Characteristics	Points			
		ICGG0	ICEM 0.3	ICEM0.5	ICEM 1.0
1	Flavor	8.4	7.6	**8**	7
2	Color	7.8	7.4	**8.2**	7.2
3	Texture	8.4	7.2	**8.4**	7.2
4	Appearance	7.8	7.6	**8.4**	7.4
5	Taste	8	6.8	**7.8**	7
6	Mouth feel	8.4	6.8	**8.2**	7
7	After taste	7.8	6.6	**8.2**	6.8
8	Overall Acceptability	8.09	7.14	**8.17**	7.09

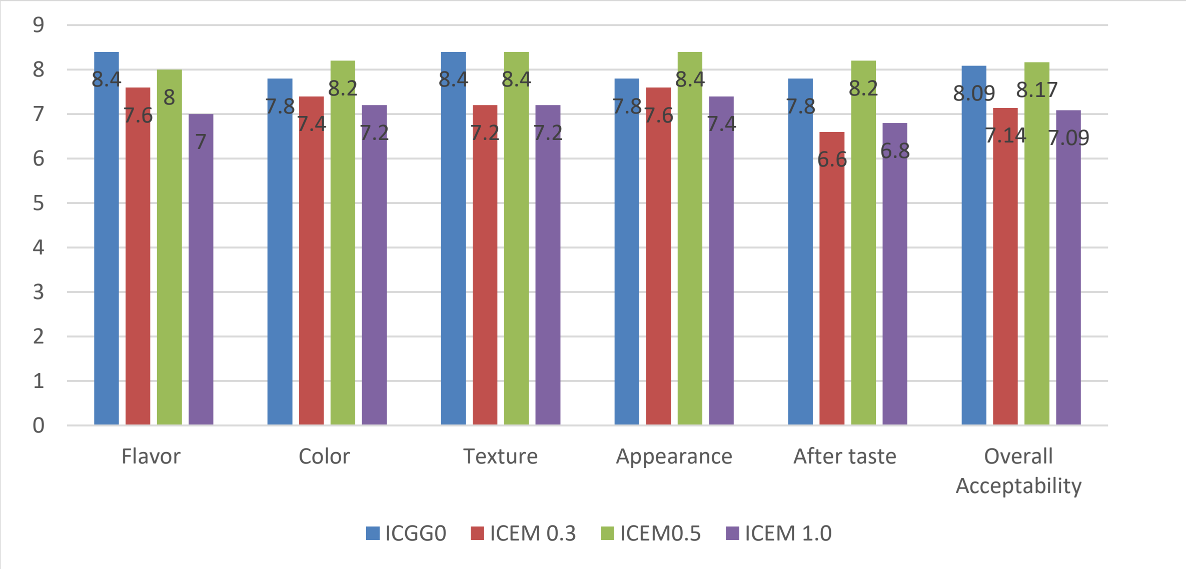


Figure 2: Sensory hedonic chart of ICEM

Sensory evaluation of formulations results the stability of ice cream in all the samples (ICEM 0.3, ICEM 0.5 and ICEM 1.0) was better than control sample referred in literature.

In all the EMTS, enriched ice cream there was less smoothness than control sample but this might be because of improper whipping of cream or power fluctuation of freezer. Apart from this in the samples ICEMC 1.0 there was starch aftertaste remain in mouth which makes formulation unacceptable.

In sample ICEM 0.5 there was texture, stability, taste and mouth feel are better than control sample so the sample ICEM 0.5 formulated product was reported as best.

3.4 Chemical composition of Ice cream

TABLE VI
COMPOSITION OF ICE CREAM

Sr. No.	Composition	ICGG0	ICEM-0.5
1.	Moisture (%)	88.36±2.35	90.35±2.41
2.	Fat (%)	6.77±1.32	5.25±1.65
3.	Protein (%)	2.36±0.75	2.69±0.87
4.	Total solids (%)	34.55±2.22	35.82±2.48
5.	Ash (%)	0.77±0.09	0.45±0.21

3.5 Physical properties of Ice Cream

TABLE VII
VISCOSITY AND OVERRUN OF ICE CREAM

Sr. No.	Properties	ICGG	ICEM 0.5
1.	Viscosity (Cp)	92.72±1.16	110.21±1.05
2.	Overrun (%)	62.31±2.35	65.67±2.57

Viscosity and Overrun of ice cream mixes containing commercial stabilizer like Guar gum and EMTS are shown in Table VII. The results had been demonstrating that the viscosity of ICES0.5 was higher than that of ICGG. Overrun of modified formulations is comparatively more than control sample which means that the EMTS improves the overrun of ice cream.

Results also indicated that the Enzymatically modified taro starch will be better replacer for guar gum and might be other commercial stabilizers.

00 Min 30 Min 60 Min 90 Min 120 Min

00 Min 30 Min 60 Min 90 Min 120 Min

Figure 3: foams stability of ice cream formulations

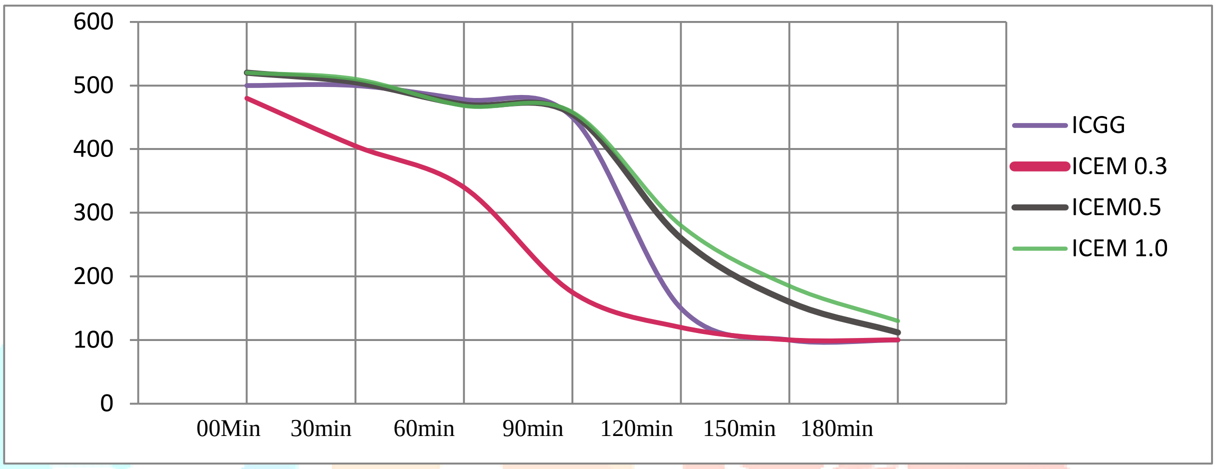


Figure 4: Foam stability of ice cream mixes ICGG, ICEM0.3, ICEM0.5, ICEM1.0

The foams stability of ice cream in formulated ice cream mixes were determined. The results showed that foams of ice cream mixes comprising ICEM 0.5 and ICEM 1.0 were the most stable with guar gum, while foams of the mixe (ICEM 0.3) rapidly decreased in the duration time from 0 to 120 min. In ICEM 1.0 stability was high but this formulation was fail in sensory evaluation so this was not acceptable. In comparison with guar gum sample ICEM 0.5 was better. After 90min guar gum stabilized ice cream mix suddenly decreases the stability while in ICEM 0.5 mix was more stable.

Results suggested that the viscosity of formulations contributed its role. The higher the viscosity of ice cream mixes, the more stability of foams, since air bubbles break in the ice cream was limited. This resulted in less air bubbles break then lesser the foam collapse. However, the foam stability of all ice cream mixes decreased with increasing time of observation.

IV CONCLUSION

Study concludes that EMTS prepared by treating the taro starch by α-amylase resulted in improvement of functional properties Viz. Swelling power (18.28 g/g) and enzyme digestibility (62.7%) which were higher than that of extracted starch Swelling power (17.43 g/g) and enzyme digestibility (59.32%). It was also noticed that the 0.5% incorporation of EMTS resulted in improvement in the ice cream stability and overall quality with improved viscosity (110.21Cp) and overrun (65.67%**)** which might be due to high swelling or gas retention capacity of α-amylase modified taro starch.

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