



## **Study of Soup Mix Incorporated with Starch Extract from Mango “*Mangifera indica*” Seed Kernels**

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### **Abstract**

Mango processing generates kernels and peels as waste which accounts for 40-60 per cent. Mango kernels most often used as by-product for extraction of oil. The residue left after oil extraction is generally considered as waste but studies revealed that it has sufficient amount of starch. Present study was undertaken to extract starch from mango seed kernels, to investigate level of incorporation of starch extract into soup mix and to study its shelf life. Mango seeds were procured from processing industry and kernels were isolated manually from its seed coat. Starch was extracted from kernels. Extracted starch was incorporated in soup mix by replacing corn starch at 25, 50 and 75 per cent. Best accepted sample was kept for storage study in comparison with control for two months. Results revealed recovery of starch was 50.5 g per 100 g of kernels. Starch extract incorporated soup mix was best accepted at 50 per cent level and this sample was kept for storage study in comparison with control soup mix where 100 per cent corn starch was used. Over the storage period sensory scores for soups prepared with soup mixes were observed to be acceptable indicating two months of shelf-life for soup mix. Microbial load on starch extract incorporated soup mix was observed to be less when compared to control but on both samples it was observed to be within safety limits. Mango kernels which are generated as processing waste can be utilized to extract starch, which can replace the corn starch up to 50 per cent in soup mixes without significant difference in sensory properties and can be stored for two months.



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### **Introduction**

Among the important tropical fruits, Mango “*Mangifera indica*” is the main fruit of Asia which has developed

its own importance throughout the world. It is one of the important fruit in Indian fruit basket accounts for 36 per cent to total fruit area, 20.3 per cent to

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total fruit production. Mango processing generates 40–60 per cent of waste, where peel and kernel contribute 12–15 and 15–20 percent, respectively.<sup>1</sup> Disposal of this waste may cause environmental hazards which may further be elevated when subjected to legal restrictions. In recent years thus new concepts of considering the use of these processing waste as by-products for further utilization and extraction of useful components from them have developed increasing interest.<sup>2</sup> Various studies have revealed that by-products generated from fruits and vegetable processing are good sources of potentially valuable bioactive compounds. There are reported studies on utilization of mango stone in replacement of wheat flour or for extraction of edible oils. The kernel obtained after decortications of mango stone is good source of starch, fat and protein. Starch has got its important applicability in food and pharmaceutical industry. Due to its wide applicability various industries depend upon crops that are the primary source of food for the human, causing increase in food prices. Recently, isolation of starches from fruit seeds which are generated as by-products after utilization of pulp, were conducted and their composition was also characterized. In mango processing seeds which are generated as waste are generally exploited for its oil extraction. A study reported that, the residue left after oil extraction from kernel generally termed as “total waste” has adequate amount of starch.<sup>1</sup> Starch extracted from mango kernel is of good quality and there is difference in physicochemical properties of starch from different varieties of mango kernel suggests its application for both food and non-food purposes.<sup>3</sup> Starch as an additive in food processing finds its use as thickeners and stabilizers in processed foods such as puddings, custards, soups, sauces, gravies, salad dressings, and to make noodles and pastas. Among these processed foods, application of starch in soups is very extensive, both in freshly prepared and dry soup mix. Soup is generally a liquid heterogeneous food category, predominantly served hot, which is prepared using vegetables or meat with stock, juice or water with thickening agents. Some dry instant soups are prepared with thickening ingredients, such as pregelatinized starch. Various studies have reported on different methods of starch extraction from mango kernel, its physicochemical and structural properties. But there are limited studies

available on applicability of extracted starch from mango kernel to food products. The present study was undertaken with the objective to extract starch from mango seed kernel, to investigate its level of acceptability in soup mix and to study its shelf-life.

## **Materials and Methods**

### **Procurement of Mango Seed Sample**

Seed waste of Totapuri variety fruit was procured from a local mango processing, Safal industry, Bengaluru, Karnataka, India

### **Total Starch Content Determination**

Total starch content of kernels was determined with standard protocol.<sup>4</sup> Soluble sugars were extracted with ethanol (95%) and residual starch was then hydrolysed with perchloric acid into monosaccharides. The sugar was colorimetrically determined with phenol-sulphuric acid.

### **Extraction of Starch from Mango Seed Kernel**

The recovered kernels were cut into small pieces and steeped in 0.16% aqueous solution of sodium hydrogen sulphite for 24 hrs at 50°C for preservation. The solution was decanted, and the samples were ground in a laboratory blender. The ground slurry was screened through muslin cloth where it was left and washed thoroughly with distilled water. After 1 hr, the supernatant was decanted from the filtrate and the settled starch layer was re-suspended in distilled water and centrifuged at 2800 rpm for 5 min. The upper non-white layer of the residue was scraped off and the white layer was re-suspended in distilled water and centrifuged. This was repeated 4 more times and finally the starch was then collected and dried at 50°C for 6 hours in an oven 5 ( Fig.1).

### **Nutrient Analysis**

Extracted starch was analysed for its moisture, protein, fat, crude fiber and ash with standard protocols.<sup>5</sup> Each nutrient was analysed in triplicates and mean values are represented in table 1. Carbohydrate was calculated by difference method and energy value was computed with energy yielding nutrients.

### **Functional Properties**

Starch extract and corn flour were studied for functional properties viz., bulk density, water

absorption capacity, oil absorption capacity, swelling power and solubility index in triplicates and mean values are represented in table 2.

**Bulk Density**

The sample (50 g) was put into a 100 ml graduated cylinder and tapped 20-30 times. The bulk density was calculated as weight per unit volume of sample.<sup>7</sup>

**Water and Oil Absorption Capacity**

One gram sample was mixed with 10 ml of distilled water or with 15 ml oil for 30 minutes. The contents were allowed to stand at 30°C in a water bath for 30 minutes and then centrifuged at 3000 to 5000 rpm for 20 to 30 minutes. After centrifuging the volume of the supernatant was recorded and used for determination of water and oil absorption and the results were expressed as ml/g sample.<sup>8</sup>

**Swelling Power and Solubility Index**

About 0.4 g of sample in a centrifuge tube was treated with 40 cm<sup>3</sup> of distilled water. The slurry was heated at 0, 50, 60, 70, 80, 85 and 90 °C in a water bath for 30 min. After cooling to room temperature, the solution was centrifuged at 3000 rpm for 15 min. The supernatant was carefully recovered, and the swollen starch sediment was weighed.<sup>5</sup>

Aliquot of the supernatant was then evaporated overnight (110°C) and swelling power and solubility were calculated from equations (1) and (2).

$$\text{Swelling power (g / g)} = \frac{\text{Weight of the wet sediment (g)}}{\text{Weight of the dry sample (g)}} \dots(1)$$

$$\text{Solubility (\%)} = \frac{\text{Weight of dry supernatant (g)}}{\text{Weight of the dry sample (g)}} \times 100 \dots(2)$$

**Determination of Amylose and Amylopectin Contents**

0.10g of the sample was weighed into a 100 cm<sup>3</sup> volumetric flask and 1 cm<sup>3</sup> of 99 per cent ethanol and 9 cm<sup>3</sup> of 1 M sodium hydroxide solution were carefully added. The contents were mixed thoroughly and the sample solution was heated for 10 mins in boiling water to gelatinize the starch. After cooling the solution was made up to the mark with distilled water and shaken thoroughly. Five (5) cm<sup>3</sup> of the starch solution in a 100 cm<sup>3</sup> volumetric flask was treated with 1.0 cm<sup>3</sup> of 1M acetic acid and 2.0 cm<sup>3</sup> of iodine solution. The solution was diluted to the mark with distilled water and the absorbance was read using spectrophotometer at 620 nm. Absorbance of blank solution prepared accordingly was subtracted from that of the sample and amylose and amylopectin contents were calculated using equations (1) and (2) respectively:

$$\text{Amylose content (\%)} = 3.06 \times \text{absorbance} \times 20 \dots(1)$$

$$\text{Amylopectin (\%)} = 100 - \% \text{ amylose content} \dots(2)$$

**Development of Soup Mix with the Incorporation of Starch Extract**

Instant Soup mix was developed and standardized with ingredients generally used in soup mix preparation. Corn starch was the main ingredient in soup mix, used as thickening agent which was replaced by extracted starch at 25, 50 and 75 per cent levels per serving size (Fig 2)



**Fig. 1: Extraction of starch from mango seed kernels**

Mix all the ingredients (Salt, sugar, maida and milk powder) and dried spices (onion, garlic, ginger and pepper)

mix was evaluated for sensory scores and microbial load during, initial, 30 and 60 days of storage period.

**Sensory Evaluation of Soup Mixes**

Soup was prepared by soup mixes containing different levels of starch extract and subjected for sensory evaluation (Subjective method). Acceptance preference test was employed to evaluate parameters like appearance, consistency, taste, colour, aroma and overall acceptability of soups on nine point hedonic scale 9 by trained panel members (n=21).

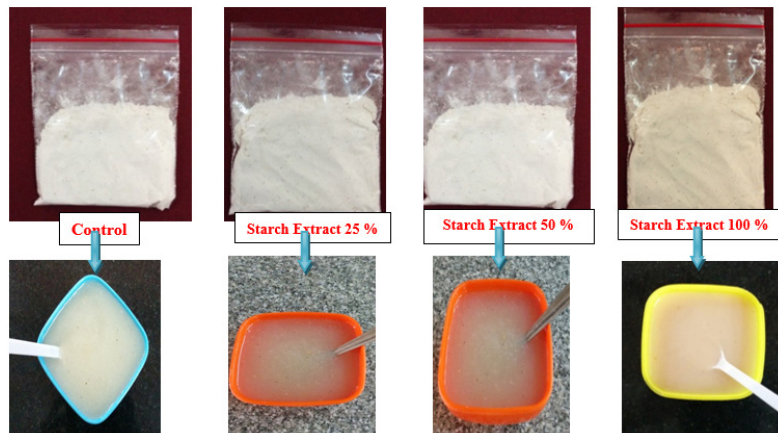
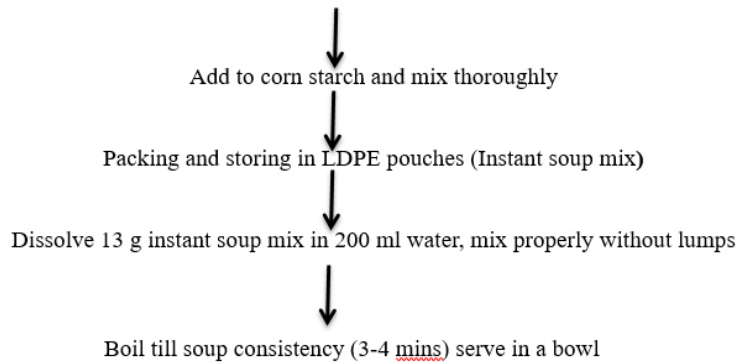
**Microbial Study of the Food Products**

The microbiological analysis of the developed soup mix samples was carried out as per the standard method for coliforms using the Eosine Methalene Blue Agar (EMBA), for general bacteria nutrient agar and for fungi using Rose Bengal Agar (RBA). Ten gram of sample was diluted in 90 ml of buffer blanks and subsequent dilutions were prepared up to 10<sup>-6</sup> dilution. Three dilution factors were used in triplicates for plating of coliforms, general bacteria and fungi viz., 10<sup>-2</sup>, 10<sup>-3</sup>, 10<sup>-4</sup>, 10<sup>-5</sup> and 10<sup>-6</sup>. The number of microbial counts was calculated using the following formula.<sup>10</sup>

**Shelf Life Study of the Products**

After sensory evaluation the best accepted level of incorporation of starch extract into soup mix and control were packed in food grade 300 gauge LDPE (low density polyethylene) pouches to conduct storage study. Soup mix packed in LDPE pouches was stored at room temperature for 2 months. Soup

$$\text{No. of Microorganisms (per g/ml)} = \frac{\text{No. of colonies} \times \text{dilution factor}}{\text{Weight / volume of aliquots taken (g/ml)}}$$



**Fig. 2: Instant Soup Mix Incorporated with Starch Extract at Different Levels**

### Statistical Analysis

The data was tabulated keeping in view the objectives of the study. One way analysis of variance (F-test) was applied for sensory characteristics to test the significant difference between samples in the study. Student t- test was done to compare functional properties of samples. Two way analysis of variance (F- test) was used for sensory scores of the products on storage. Significant difference was defined at  $p \leq 0.05$  (5%).<sup>10</sup>

### Results and Discussion

#### Starch Extraction

Starch is the predominant food reserve substance in plants. It is greatly in abundance and is readily converted into useful products. Thus, it finds application in many industries. In the present study starch content in mango kernel was found to be 68.50 percent. Recovery of starch was 50.5 g per 100 g of kernels (73.7 %). Similar findings observed and reported starch content ranged between 52.80 to 65.37 percent, indicating varietal difference.<sup>5</sup> Some findings observed to be lower, 55 and 46.69 per cent for starch and its recovery respectively.<sup>12</sup> In present study solid solvent ratio of 1:10 was used. Reported study which examined influence of solid to solvent ratio revealed, yield of starch increases as the solid to solvent ratio increases and maximum yield was observed for 1:14 solid to solvent ratio.<sup>3</sup> Isolation of starch will not be effective at low solid to solvent ratio. Whereas when higher solid to solvent ratio is used kernels are steeped properly and isolation of starch will be effective and recovery observed to be high. Recovery of starch is dependent on the ratio of solid to solvent used in the extraction method.

#### Nutrient Composition of Starch Extract

Nutrient composition of extracted mango kernel starch is presented in Table 1. Results showed that moisture, protein, fat, crude fiber, carbohydrate, ash and energy content of starch extract was 10.41 percent, 4.86, 3.64, 0.32, 80.72, 0.37 g and 374 k. cal. per 100 g respectively. Nutrient composition of starch extract indicates presence of fat and protein. There are reported lower values for all the nutrients in extracted starch of mango kernels (5). In the present study since no alkali treatment was given during starch extraction, protein was not completely separated.

### Physicochemical Properties of Corn Starch and Mango Kernel Starch Extract

In present study physicochemical properties of kernel starch extract was compared with the corn starch as it was used in control soup mix sample as reference and which is one of the common ingredients in commercial soup mixes as thickening agent. Results of physicochemical properties are presented in Table 2. Statistical analysis with student t-test shows that except for bulk density and gelatinization temperature, all other parameters had significant difference at 5 percent level between the corn starch and starch extract from kernel.

Starch extract had lower water and oil absorption capacity compared to corn starch. It was found to be 2.10 and 1.20 ml per g for water and oil absorption capacity respectively. Corn starch had more water absorption (2.8 ml/g) and oil absorption (1.7 ml/g) capacity compared to starch extract from kernels. Studies reported lower water binding capacities (WBC) of starches from mango kernel cultivars (89.5 to 97.7%) than starches from potato cultivars. The main structural components of starch viz., amylose and amylopectin, their loose association may be responsible for reduced water and oil absorption capacity compared potato starches<sup>1</sup>. A study compared impact of different drying method on water and oil absorption index for kernel powder, reported significant difference between drying methods employed, which ranged from 1.83 to 2.24 (g/g) and 1.69 to 2.30 g/g for water and oil absorption index respectively. Cabinet dried sample had lowest indices compared to other methods (Freeze, vacuum and IR)<sup>12</sup>. These range of findings

**Table 1: Nutrient composition of starch extract per 100 g (n= 3 replications)**

Nutrients	Composition
Moisture (%)	10.41±0.12
Protein (g)	4.86±0.21
Fat (g)	3.64±0.10
Crude fiber (g)	0.32±0.06
Carbohydrate (g)	80.72±0.37
Energy (K.cal)	374±0.23
Ash (g)	0.37±0.03

observed to be on par with results of present study. Use of cabinet drier in present study may be responsible for low water and oil absorption capacities of starch extracts compared to corn starch. Since starch is the major component of mango seed kernel these findings can be correlated. Morphology of starch granules, structural differences, size of starch molecules and association between starch granules showed greater impact on water and oil absorption capacities.<sup>13</sup> There are reported studies on structural difference between corn starch and mango kernel starch, attributed to difference in functional properties of study samples.

Solubility index of starch extract (14.9 %) was high compared to corn starch (11.3 %) whereas swelling

power of corn starch (12.3 g/g) was more than starch extracts (8.2 g/g). Swelling power of starches of different mango cultivars ranged from 18.0 to 19.7 g/g and solubility index ranged from 0.141 to 0.149 g/g (1). These findings are in supportive to the present results for solubility index. Higher values are reported for swelling power. Magnitude of interaction between two predominant chains of starch granules, amylose and amylopectin has influence on swelling power and solubility index of starch. Extent to which these two chains interact depends on several characteristics such as, amylose/ amylopectin ratio, molecular weight distribution and length of branching. Varietal difference in mango attribute to difference in these characteristics leading to changes in functional properties<sup>14</sup>.

**Table 2: Physico-chemical properties of corn flour (CF) and starch extract used in development of instant soup mix (n=3)**

Sample	Bulk Density (g/ml)	Water absorption capacity (ml/g)	Oil absorption Capacity (ml/g)	Swelling power (g/g)	Solubility Index(%)	Gelatinization Temperature (°C)	Starch Content (%)	Amylose (%)	Amylopectin (%)
CF	0.57±0.01	2.80±0.1	1.70±0.1	12.30±0.1	11.30±0.2	64.0±1.0	84.0±1.0	17.20±0.2	82.80±0.2
Starch extract	0.59±0.01	2.10±0.1	1.20±0.1	8.20±0.1	14.90±0.5	66.0±1.0	80.0±1.0	14.60±0.6	85.40±0.6
t-Value	NS	7.00*	5.00*	41.00*	8.48*	NS	4.00*	5.36*	5.36*

NS - Non Significant \* Significant at 5% level

**Table 3: Mean sensory scores of instant soup mix incorporated with starch extract (n=21)**

Level of Incorporation	Appearance	Consistency	Colour	Aroma	Taste	Overall acceptability
Control	8.68 <sup>a</sup>	8.48 <sup>a</sup>	8.50 <sup>a</sup>	8.65 <sup>a</sup>	8.58 <sup>a</sup>	8.56 <sup>a</sup>
Starch extract 25 %	8.65 <sup>a</sup>	8.50 <sup>a</sup>	8.35 <sup>a</sup>	8.38 <sup>a</sup>	8.32 <sup>a</sup>	8.41 <sup>a</sup>
Starch extract 50 %	8.63 <sup>a</sup>	8.51 <sup>a</sup>	8.40 <sup>a</sup>	8.40 <sup>a</sup>	8.35 <sup>a</sup>	8.42 <sup>a</sup>
Starch extract 100 %	7.90 <sup>b</sup>	7.50 <sup>b</sup>	7.40 <sup>b</sup>	7.40 <sup>b</sup>	7.38 <sup>b</sup>	7.40 <sup>b</sup>
F – Test	*	*	*	*	*	*
SEm±	0.11	0.11	0.11	0.12	0.12	0.32
CD at 5 % level	0.31	0.32	0.32	0.35	0.33	0.91

NS – Non Significant \* Significant at 5% level (Means in the same columns followed by different letters are significant)



Starch, amylose and amylopectin content for corn starch were 84.0, 17.2 and 82.8 respectively and for mango kernel starch it was 80.0, 14.6, and 85.4 percent. All these parameters significantly differed at 5 per cent level between test samples. Amylose content of kernel starch extract was lower compared to corn starch. Amylose content of starches of different mango kernel cultivars ranged from 9.1 to 16.3 percent.<sup>1</sup> These findings support the results of present study. Large-size granules in case of corn starch responsible for high amylose content. Whereas small size granules of mango kernel starch as reported by the studies may attribute to its low amylose. Findings of starch, amylose and amylopectin of starch extract are in line with the results reported for different varieties of mango kernel flour.<sup>5</sup>

**Sensory Evaluation of Soup Mix**

Statistically significant difference at 5 percent level existed for all the sensory parameters between control and starch extract incorporated soup samples for all the sensory attributes (Table 3). Replacement of corn starch with starch extract up to 50 percent per serving had sensory scores on par with control, showing statistically non significant difference at 5 percent level, whereas further incorporation level significantly lowered the scores. Incorporation of mango kernel starch was acceptable up to 50 percent with sensory scores on par with the control. But when only starch extract was used (100% incorporation) sensory scores decreased significantly, which may

be attributed to the functional properties of starch extract. As the level of incorporation of starch extract increased major changes were observed in consistency and appearance of soup. Overall acceptability scores were high for control (8.56) soup, followed by 50 percent (8.42) and 25 per cent (8.41) starch extract incorporated soup. Among variations, 50 percent starch extract incorporation was best accepted and was kept for shelf life study. Replacement of 50 percent of watermelon seed flour in egusi (melon seeds) soup which is popular in Nigeria, had non-significant difference between sensory scores when compared to original egusi soup prepared with 100 per cent melon seed flour.<sup>15</sup> These findings support the incorporation of seed flours in soups is acceptable at 50 percent level of incorporation.

**Effect of Storage on Sensory Scores of Instant Soup Mix**

Soup mix of control and 50 per cent starch extract incorporated samples were studied for a period of 2 months (60 days) for sensory scores. Soup mixes were sealed in LDPE (low density polyethylene, 300 gauze) bags and kept at ambient temperature and evaluated periodically (initial, 30<sup>th</sup> and 60<sup>th</sup> days) for sensory attributes. Fresh soup was prepared and evaluated by panel members and the results are presented in Table 4.

Results showed that, except for appearance, all the mean sensory scores were significantly

**Table 5: Microbial load of instant soup mix during shelf life study (per g) (n=3)**

Products	Storage period								
	Initial			30 days			60 days		
	Total bacterial count (CFU/g)	<i>E.Coli</i> (CFU/g)	Fungi (CFU/g)	Total bacterial count (CFU/g)	<i>E.Coli</i> (CFU/g)	Fungi (CFU/g)	Total bacterial count (CFU/g)	<i>E.Coli</i> (CFU/g)	Fungi (CFU/g)
<b>Instant Soup mix</b>									
Control	1.0X10 <sup>3</sup>	Nil	Nil	1.3X10 <sup>3</sup>	Nil	Nil	2.0X10 <sup>3</sup>	Nil	Nil
Starch extract 50 %	9.6X10 <sup>2</sup>	Nil	Nil	9.8X10 <sup>2</sup>	Nil	Nil	1.4X10 <sup>3</sup>	Nil	Nil



differed between control and starch extract (treatment) incorporated soup. Sensory scores were significantly reduced with duration (60 days), but were observed to be within acceptable range, according to the sensory evaluation score card. Interaction of treatment (incorporation of starch extract) and storage period was also observed to be significant for all the sensory attributes, except for consistency scores.

Sensory scores significantly decreased along two months (60 days) of storage period for instant soup mix. Incorporation of starch extract has also lowered the sensory scores compared to control over the storage period. Soup mix was a dry mix of mainly corn flour and starch extract with spices, without any heat treatment, which may attributed to decrease in sensory scores of soup mixes. Nutrient composition of starch extract reveals presence of fat, oxidation of which may attribute to lower the sensory scores when compared to control soup mix. At the end of storage period, overall acceptability scores of control and 50 percent starch extract incorporated soup was 8.45 and 8.20 respectively (Table 4). Which indicated the acceptability of both the products at 60<sup>th</sup> day (2 months) of storage period. Soup prepared by dehydrated vegetable mixtures had the shelf life of 3 months period with overall acceptability score of 6.23 at the end of storage period.<sup>16</sup> These outcomes are in line with present study.

#### Microbial Load of Developed Food Products during Storage Period

Control instant soup mix had the TBC (Total Bacterial Count) of  $1 \times 10^3$ ,  $1.3 \times 10^3$  and  $2 \times 10^3$  CFU per g at initial, 30 and 60 days of storage period respectively. Starch extract incorporated soup mixes had TBC at initial ( $9.6 \times 10^2$  CFU), during 30 ( $9.8 \times 10^2$  CFU) and 60 days ( $1.4 \times 10^3$  CFU) of storage period. Mold counts were nil in soup mixes. Total bacterial counts were observed from initial to 60 days (2 months) of storage period as no heat treatment involved in

its dry mix preparation. But it was observed to be within safe limits. Control had more TBC compared to starch extract soup mix at all storage periods. Lower microbial load on starch extract soup mix may be attributed to its bound polyphenol content (8 mg GAE/ g) which has antimicrobial property (table not presented). A study reported aerobic plate count of the fresh vegetable soup mix as  $2.1 \times 10^3$  cfu/g and for prawn added soup mix  $2.9 \times 10^3$  cfu/g after 2 months of storage period.<sup>17</sup> Product is microbiologically safe if the total microbial count of dehydrated soups is less than  $1 \times 10^4$  cfu/g.<sup>18</sup> These findings support the results for microbial load on instant soup mixes over storage period and their shelf life.

#### Conclusion

During processing of ripe mango, its seeds are generated as waste. This waste is a problem for canning factories as its disposal may appreciably increase environmental pollution. Mango seed kernel which is generated as waste can be effectively treated as by product to extract starch. When replaced for corn starch in soup mix, this starch extract from mango seed kernel found to be acceptable till 50 percent incorporation level, with shelf life of up to two months. Further different methods to extract starch from mango kernel to increase its recovery can be explored and molecular study of the starch granule also finds scope to understand various properties of mango kernel starch helpful to widen its application in food processing and to study its health implications.

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