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Preparation and quality evaluation of beverage from a blend of apple pulp and WPC

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Abstract
The present study was carried out to prepare apple pulp and Whey Protein Concentrate **(**WPC) based beverage by incorporation of skim milk, WPC, apple pulp, sugar and stabilizer with the objective to assess the chemical properties and sensory acceptability of the product. Experimental treatments were prepared by varying the level of apple pulp i.e. 5% (T_1), 10% (T_2), 15% (T_3) and 20% (T_4). Each treatment was replicated five times. Sensory evaluation of the product was carried out using 9 point hedonic scale. The data obtained during the study was analyzed statistically using variance and critical difference techniques. It was concluded that T_3 (15% apple pulp, 2% WPCs, 3% sugar and 0.1% stabilizer with skim milk) was found to be best organoleptically among the four treatments. The percent acidity, fat, protein, carbohydrates, ash and total solids in T_3 were found to be 0.29, 0.46, 5.40, 14.14, 0.85 and 21.14 respectively. The results revealed less than 50/ml (standard value) SPC and coliform test was nil. Hence the product was acceptable, thus apple pulp can be successfully incorporated upto 15% level in WPC apple pulp based beverage.

Keywords: apple pulp; beverage; quality evaluation; skim milk; WPC

1. Introduction
Whey based beverage production started in 1970's and until today a wide range of different types of whey beverages has been developed. Whey constitute about 80-90% of the volume of milk used for the conversion into channa, paneer, cheese and casein. By realizing the functional properties of whey, many industries targets upon utilizing whey as the functional food ingredient. Whey protein concentrates, whey protein isolates and whey powder is prepared and widely marketed all over the world but all these process need particular expensive equipments. Hence the conversion of whey into beverage is one of the most attractive ways for utilizing whey for human consumption (Goyal and Gandhi, 2009) [7].

Whey proteins are good in taste, versatile and highly functional ingredients and therefore provides essential benefits to food and beverage manufacturers as they create nutritious and delicious products that consumers are demanding. Whey proteins provide highest quality absorbtion characteristics as well as sulphur containing essentially branched chain amino acids like leucin, isoleucine and valine which are important in growth and repairing of tissue, improve muscle strength and body composition (Khare *et al*., 2007) [13]. Whey proteins referred as "fast protein" for its ability to quickly provide nourishment to the muscles. Commercial sport drinks, health drinks, fermented whey drinks and non fermented whey beverages are available in the market under different brands (Goyal and Gandhi, 2009) [7].

Apple is one of the most popular fruit in the world. "An apple a day keeps the doctor away" is an old Welsh proverb the most of us familiar with (www.worldatlas.com). It is one of the most cultivated and consumed fruits in the world. Apple is continuously being praised as a "miracle food". It promotes heart health in several ways (www.medicalnewstoday.com). It is good source of vitamin C and potassium. It contains polyphenols which can have numerous health benefits (Sun *et al.*, 2002) [20]. Apple is high in soluble fiber, which helps lower cholesterol. It also has several naturally occurring compounds that may help to fight cancer (Leontowicz *et al*., 2002) [15]. It contains antioxidant and inflammatory compounds that help to regulate immune responses and protect against asthma. An analysis of studies found that high in takes of flavonoids were linked to a 20% lower risk of stroke. Apple juice also helps prevent the decline of neuro-transmitters that are involved in maintaining healthy memory (Boyer and Liu, 2004) [2].

Ninety-percent of the energy from apples is derived as carbohydrates, mainly sugars; of which fructose is in the dominant form (Dray *et al*., 2009) [4]. The fibre content of apple is approx. 3 g/100 g fresh weight (FW) and consists mainly of soluble fibres like pectin (Li and Komarek, 2017) [16]. Pectin is a complex polysaccharide, and apple pectin exhibits a high degree of esterification and a particularly high content of branched side chains. Pectin exerts different physiological roles. It exerts probiotic effects and is fermented by the micro flora in the large intestine resulting in the formation of short chain fatty acids (SCFA) which are absorbed and metabolised in the colonic mucosa, liver or peripheral tissues. It has been established a relationship between the consumption of pectin and maintenance of normal blood cholesterol concentrations and a reduction of post-prandial glycaemic responses (Wasnik, 2016) [22]. Nutritional Value of apple per 100 g - Energy 52 kcal, Carbohydrates 13.81 g, Sugar 10.39 g, Dietary fiber 2.4 g, Vitamins C 6% (4.6 mg) and Potassium 2% (Kazii *et al*., 2015) [11] and polyphenols like flavan-3-ols (0-3.4 mg/100 g) and phenoloic acids (3-43 mg/ 100 g) etc. with antimicrobial properties (Giomaro *et al*., 2014; Alberto *et al*., 2006) [5, 1].

Considering the requirements for healthy nutrition for Indian population, a WPC and apple pulp enriched dairy drink was developed. This work was taken to study the combined effect of different levels of apple pulp, 2% WPC, 3% sugar, 0.1% guar gum and 100 ml skim milk on the physico-chemical, microbiological and sensory attributes of ready to serve (RTS) beverage.

2. Materials and Methods

Materials

Skim milk was procured from the student training dairy plant of Warner College of Dairy Technology, SHUATS. WPC of 75% protein percentage with brand name 'My Protein' was used as an ingredient for increasing protein percentage in the product. It was collected from local market of Allahabad. Sugar and fresh apple fruits were collected from the local market of Allahabad. Stabilizer (guar gum) was collected from Warner College of Dairy Technology, SHUATS laboratory.

Methods

The beverage was prepared using WPC (2%), Sugar (3%), Stabilizer (0.1%), Skim milk (100 ml) and varying levels of apple pulp (T_1 5%, T_2 10%, T_3 15 %, T_4 20%). All the treatments were replicated five times and mean values were obtained for each result. The experiment comprised of 20 trials. Analysis of variance (ANOVA) was conducted for fitting the models and to examine the statistical significance of the model. The sensory properties of the beverage were judged by panel of seven judges using 9 point Hedonic scale for flavor, colour and appearance, body (consistency) and over all acceptability.

Treatment combinations

- T_1 Beverage prepared with the addition of 5% apple pulp+2% WPC+3% sugar+0.1% stabilizer.
- T_2 Beverage prepared with the addition of 10% apple pulp +2% WPC+3% sugar+0.1% stabilizer.
- T_3 Beverage prepared with the addition of 15% apple pulp +2% WPC+3% sugar+0.1% stabilizer.
- T_4 Beverage prepared with the addition of 20% apple pulp +2% WPC+3% sugar+0.1% stabilizer.

Microbiological analysis

Standard plate count

The total numbers of viable bacteria in the protein enriched beverage made from apple pulp and WPC were estimated by the method described by Houghtby *et al*. (1992) [8] by using Plate Count Agar (pH-7.0±0.1) as a nutrient medium. The prepared plates were incubated at 37 ^{0}C for 48 hours. After completing the period of incubation the results were observed for appearance of colonies and were counted. The results were expressed as log cfu per ml of sample as Log cfu per ml = log (Average no. of colonies x dilution factor)

Coliform count

Total coliform (cfu/ml) counts in protein enriched beverage were determined as per the standard procedure described in IS: 5401 (1969) [9] using violet red bile agar. The plates were incubated at 37±0.5 ^{0}C for 18 to 24 hours followed by enumeration.

Yeast and mould count

Yeast and mould counts (cfu/ml) in whey protein enriched beverage were determined as per standard procedure described in IS: 5401 (1969) using Potato Dextrose Agar. The plates were incubated at 30±0.5 ^{0}C for 2-3 days followed by enumeration.

Cost analysis

The cost of the final products has been calculated on the basis of prevailing price of raw materials.

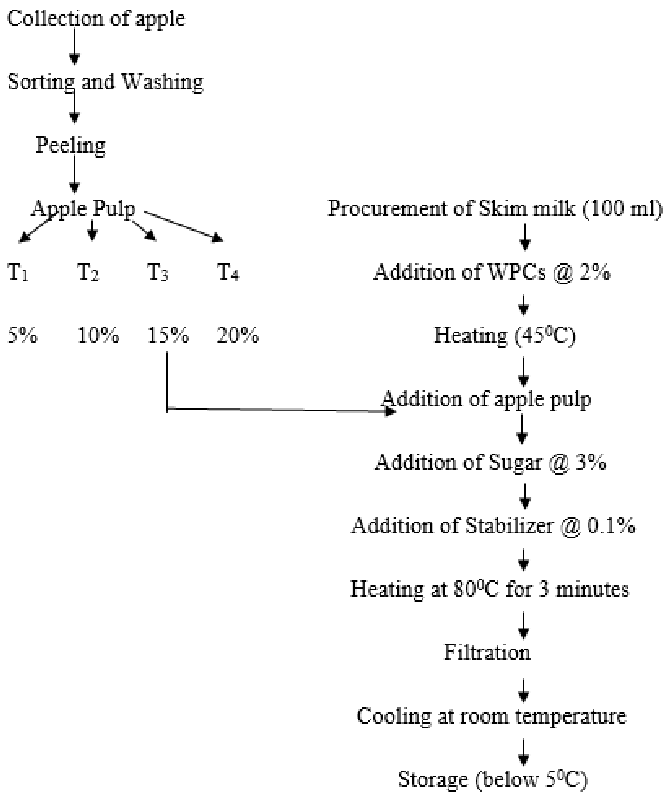

Fig 1: Flow diagram for making apple pulp and WPC based beverage

3. Result and Discussion

Chemical characteristics

Acidity

The results from Table 1 revealed that the highest mean acidity percentage was recorded in the beverage sample of treatment T_4 (0.33%) because of increasing level of apple pulp. It was followed by T_3 (0.29%), T_2 (0.26%), T_1 (0.20%). There was significant difference between all the treatments. Similar results were obtained by Jain *et al*. (2013) [10] in the whey beverage fortified with barley and Yonis *et al*. (2014) [25] in the production of whey guava beverages.

Fat

Treatment T_4 recorded for highest mean fat percentage (0.51%) in the beverage sample followed by T_3 (0.46%), T_2 (0.42%), T_1 (0.36%) as shown in Table 1. This may be due to increasing levels of apple pulp from T_1 to T_4. All the treatments differed significantly. Obtained results are in line with the findings of Jain *et al*. (2013) [10] and Shukla *et al*. (2014) [19] for the production of barley and Kiwi based whey beverages respectively.

Protein

The percent protein increased from treatment T_1 to T_4 (5.15% to 5.60%) as seen in Table 1, due to increasing level of apple pulp from T_1 to T_4 and utilization of WPC along with skim milk. There was significant difference between all the treatments. However, lesser percentages were obtained by Pareek *et al*. (2014) [18] for orange- whey beverage and Waqas *et al*. (2016) [21] for whey-pineapple beverage.

Ash

The highest mean ash percentage was depicted for the beverage sample of treatment T_4 (0.86%) followed by T_3 (0.85%), T_2 (0.84%), T_1 (0.83%) (Table 1) may be due to increasing level of apple pulp from T_1 to T_4 and incorporation of WPC and skim milk. There was non-significant difference between all the treatments. Lower levels of ash content was observed in grape juice based whey beverages (Morais *et al*., 2015) [17] whereas higher percentages were present in whey based kiwi fruit incorporated ready to serve beverages (Shukla *et al*., 2014) [19].

Carbohydrate

Data from Table1 showed the highest mean carbohydrates percent in the beverage sample of treatment T_4 (15.94%) followed by T_3 (14.14%), T_2 (12.40%), T_1 (10.56%) which can be the result of the increasing levels of apple pulp from T_1 to T_4. Significant differences were observed among all the treatments. Similar results were obtained by Jain *et al*. (2013) [10] for low cost nutritional whey beverage, Shukla *et al*. (2014) [19] for whey based kiwi fruit beverage and grape juice based whey beverages (Morais *et al*., 2015) [17].

Total Solids

As evident from the given data in Table 1, treatment T_4 noted highest mean total solids percentage (23.24%) followed by T_3 (21.14%), T_2(19.21%), T_1 (17.1%) on account of increasing levels of apple pulp. All the treatments differed significantly. Lower percentages were obtained by Pareek *et al*. (2014) [18], Shukla *et al*. (2014) [19] and Morais *et al*. (2015) [17] for whey based beverages.

Organoleptic Parameters

Flavour

The highest mean score for flavour (Table 2) was received by sample T_3 (8.360) followed by T_4 (7.5), T_2 (7.0), T_1 (6.5). The scores for treatment T_4 decline due to increased harshness. There was significant difference between all the treatments. According to Goudarzi *et al*. (2015) [6] increase in pH up to a certain value was accompanied by an improvement in flavor acceptability of whey protein-apple juice beverages, but beyond that a reduction was observed. Djuric *et al*. (2004) [3] pointed out that the balance between sucrose and pH strongly depended on the quality of whey-based fruit juice beverages. Results revealed that increasing the juice dry matter content effectively imparted a pleasant taste to whey protein-apple juice mixes.

Color & Appearance

Treatment T received the highest mean score for color & appearance was recorded (Table 2) for the beverage sample of T_3 (8.32) followed by T_4 (7.72), T_2 (7.04), T_1 (6.58). In T_4 treatment the level of apple pulp increased, simultaneously acidity increased and the product got coagulated. There was significant difference between all the treatments. As per the findings of Goudarzi *et al*. (2015) [6] apple juice dry matter content caused a remarkable enhancement of the appearance of samples. It has long been known that thermal energy can partially open the compact globular assembly of whey proteins through dissociating the intra molecular bridges exposing buried functional groups to contribute in intermolecular associations (Kazmierski and Corrdig, 2003) [12]. This forms protein aggregates that if numerous enough give an opaque appearance to thermally pasteurised whey protein- containing beverages. It has also been found that pH's far from playing a key role in the clearness of beverages because of the electrostatically repulsive forces amongst unfolded proteins or aggregated proteins/ peptides. The strong positive correlation between apple juice dry matter content and appearance acceptability can be attributed to the masking effect of apple juice on the turbid appearance of heat-treated whey protein-containing beverages. Statistical analysis revealed that the whey protein– apple juice dry matter content interaction had a significant effect ($P < 0.01$) on appearance acceptability of whey protein-apple juice mixes (Laclair and Etzel 2010) [14].

Consistency

The highest mean score for consistency was recorded for the beverage sample of T_4 (8.08) followed by T_3 (8.00), T_2 (7.08), T_1 (6.6) as seen from Table 2, with significant difference between all the treatments. As the level of apple pulp increased, percent acidity also increased from T_1 to T_4. The product got coagulated due to increasing level of apple pulp. Results obtained are in line with Goudarzi *et al*. (2015) [6].

Overall acceptability

The highest mean over all acceptability score was noted for the beverage sample of T_3 (8.14) followed by T_4 (7.50), T_2 (6.84), T_1 (6.48). There was a significant difference between all the treatments. Results showed that higher whey protein contents in the formula led to the detection of an unpleasant odour in whey protein -apple juice beverages, which hampers their overall acceptability, whereas an increase in the apple juice dry matter content donated a pleasant odour, thus improving its overall acceptability Goudarzi *et al*. (2015) [6].

Standard plate count

The highest mean SPC ($x10^3$ cfu/ml) was seen in Table 3 for beverage sample T_1 (15.26) followed by T_2 (13.20), T_3 (10.73), T_4 (8.78). The decreasing trend was observed which may be due to the presence of antimicrobial constituents like polyphenols. This is in accordance with Giomaro *et al*. (2014) [5] and Alberto *et al*. (2006) [1]. There was significant difference between all the treatments. Decreasing values of SPC from T1 to T4 may be due to increase in the total solids content which made lesser moisture available to the microbes for their growth.

Coliform count

Complete absence of the coliform ($x10^1$ cfu/ml) count was seen in all the beverage samples for treatment T_1 to T_4.

Yeast and Mold count

The result from Table 3 revealed the highest mean Yeast and mold count (cfu/ml) for T_1 (3.8) which goes on decreasing for T_2 (2.8), T_3 (1.6) and T_4 (1.6). This may be on account of presence of antimicrobial constituents like polyphenols (Giomaro *et al*., 2014; Alberto *et al*., 2006) [5, 1] in the apple pulp and also due to increased levels of apple pulp from T_1 to T_4. There was non-significant difference between all the treatments.

4. Cost analysis

It is evident from the Figure 2 that the cost of final products goes on increasing from T_1 to T_4 which may be due to increasing levels of apple pulp.

5. Conclusion

In view of the results obtained during the present investigation, it may be concluded that the apple pulp and WPC based beverage made from skim milk, WPC (2%), sugar and apple pulp (5%, 10%, 15% and 20%) was of acceptable quality. WPC increases the protein percentage and apple pulp increases the flavour and nutrients level of the product up to 15%. Treatment T_3 received highest scores in sensory evaluation and its cost of preparation was 78.0 Rs/liters.

Table 1: Effect of apple pulp and WPC on the physico-chemical parameters of the product

Parameters (%)	Treatments			
	T_1	T_2	T_3	T_4
Physico-chemical Analysis				
Fat	$0.36{\pm}0.06^a$	$0.42{\pm}0.05^b$	$0.46{\pm}0.04b^c$	$0.51{\pm}0.07^d$
Protein	$5.15{\pm}0.05^a$	$5.30{\pm}0.03^b$	$5.40{\pm}0.08^c$	$5.60{\pm}0.06^d$
Carbohydrate	$10.56{\pm}0.03^a$	$12.40{\pm}0.03^b$	$14.14{\pm}0.04^c$	$15.94{\pm}0.21^d$
Ash	$0.83{\pm}0.03^a$	$0.84{\pm}0.01^b$	$0.85{\pm}0.03b^c$	$0.86{\pm}0.02^{cd}$
Total Solids	$17.1{\pm}0.05^a$	$19.21{\pm}0.04^b$	$21.14{\pm}0.03^c$	$23.24{\pm}0.01^d$
Acidity	$0.20{\pm}0.04^a$	$0.25{\pm}0.04^b$	$0.29{\pm}0.02^c$	$0.33{\pm}0.01^d$

Data represented as means ± standard error. Means with different superscripts in a row differ significantly at 5% level of significance (n=5).

Table 2: Effect of apple pulp and WPC on sensory attributes of the product

Sensory Analysis				
Properties	T_1	T_2	T_3	T_4
Flavor	$6.68{\pm}0.21^a$	$7.36{\pm}0.15^b$	$8.36{\pm}0.12^c$	$7.48{\pm}0.11^{bd}$
Color and Appearance	$6.58{\pm}0.13^a$	$7.04{\pm}0.17^b$	$8.32{\pm}0.14^c$	$7.72{\pm}0.10^d$
Consistency	$6.60{\pm}0.10^a$	$7.08{\pm}0.07^b$	$8.0{\pm}0.09^c$	$8.08{\pm}0.13^{cd}$
Overall Acceptability	$6.48{\pm}0.31^a$	$6.84{\pm}0.35^{ab}$	$8.14{\pm}0.24^c$	$7.50{\pm}0.33^d$

Data represented as means ± standard error. Means with different superscripts in a row differ significantly at 5% level of significance (n=5).

Table 3: Effect of apple pulp and WPC on microbiological quality of the product

Microbiological Analysis				
Parameters	T_1	T_2	T_3	T_4
SPC ($x10^3$ cfu/ml)	$15.26{\pm}0.04^a$	$13.30{\pm}0.05^b$	$1.73{\pm}0.02^c$	$8.78{\pm}0.05^d$
Yeast & Mold (cfu/ml)	$3.8{\pm}0.02^a$	$2.8{\pm}0.03^b$	$1.6{\pm}0.01^c$	$1.6{\pm}0.03^c$
Coliform Count ($x10^1$ cfu/ml)	Nil	Nil	Nil	Nil

Data represented as means ± standard error. Means with different superscripts in a row differ significantly at 5% level of significance (n=5).

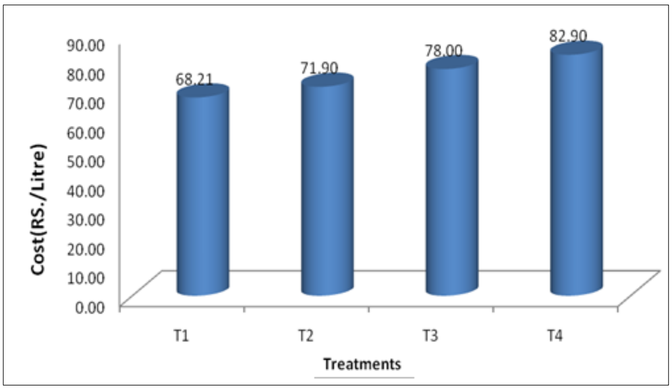

Fig 2: Cost analysis graph for for making apple pulp and WPC based beverage

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