Development of an alternative low-cost cereal-based weaning food fortified with iron and vitamin A (retinol acetate)

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ABSTRACT

The main objective of the study was to develop an alternative low-cost pearl millet-based weaning food followed by the fortification with iron and vitamin A (retinol acetate). The product was formulated as per the guidelines of Food Safety and Standards Regulations, 2010 and prepared by using extruded pearl millet flour, extruded wheat flour, skim milk powder, whey protein concentrate-70 and sucrose in the ratio of 35, 25, 15, 5 and 20%, respectively. Electrolytic iron and vitamin A acetate were fortified at the level of 12mg/100g and 360 µg/100g of the product to meet 100 and 98.25% of the RDA for iron and vitamin A, respectively. The developed weaning food was analyzed for proximate composition and physicochemical properties. The average moisture content was 3.73±0.02% and average protein, fat, carbohydrate, ash and crude fiber content (on dry weight basis) was 15.32±0.06, 1.96±0.07, 79.58±0.06, 2.02±0.02 and 1.12±0.10%, respectively. The iron, vitamin A and β-Carotene content were found to be 15mg, 393µg and 30.62µg per 100g of the product, respectively. Physical properties namely flow-ability, water absorption index, water solubility index were analyzed and values were observed to be 33.80±0.27, 9.28±0.17, 9.12±0.35%, respectively. The lightness (L*), redness (a*), yellowness (b*) values of color for the developed product were 77.87±0.26, 2.15±0.12 and 17.43±0.08, respectively. Overall, it can be concluded that the well-developed product could serve as a potential delivery system of iron and vitamin A and could be an effective tool to deal with the micronutrients deficiency in infants.

Key words: Infants, Iron, Pearl millet, Physicochemical characteristics, Vitamin A fortification, Weaning food

Nutrition in early years of life is a major determinant of healthy growth and development throughout childhood and of good health in adulthood. Pediatricians and nutritionists have established nutritional guidelines to meet specific needs of these early years. From birth up to 4 to 6 months of age, most infants are fed with breast milk, a food supply that produces adequate nutrition and some resistance to diseases (Rowland et al. 1988). After the age of 4–6 months, introduction of other foods in infants’ diet becomes necessary because human breast milk alone is not sufficient to meet the ever increasing needs of the infants (Lutter et al. 1990).

Prevalence of stunting in linear growth of children younger than 5 years has decreased during the past two decades, but is still higher in south Asia and sub-Saharan Africa than elsewhere and globally affected at least 162 million children with the vast majority living in Africa and Asia (WHO 2014). Iron deficiency and iron deficiency anaemia are major public health problems during infancy in low and middle income countries (McLean et al. 2009, Stoltzfus 2011). According to World health Organization (2014), anaemia affects half a billion women of reproductive age worldwide. In 2011, 29% (496 million) of non-pregnant women and 38% (32.4 million) of pregnant women aged 15–49 years were anaemic. India is the highest contributor to child anemia among the developing countries with approximately 89 million anaemic children (Singh and Patra 2014). On the other hand, vitamin A deficiency can lead to xerophthalmia (dryness to the eye and skin), the leading cause of childhood blindness, anaemia, and weakened host resistance to infection, which can increase the severity of infectious diseases and risk of death (WHO 2009). Deficiencies of vitamin A and iron together, can contribute to children not reaching their developmental potential. Therefore, Government of India recommends that children should be given vitamin A supplements every six months from age of nine months to three years to avoid vitamin A deficiency (Arnold et al. 2009). In such situation, fortification of weaning foods with vitamin A and iron is...
an additional tool to meet the recommendations of Government of India.

According to the FSSR (2010) guidelines, processed cereal-based complementary foods, commonly called as weaning food or supplementary food means those food which are based on cereal and/or legumes, soybean, millets, nuts, and edible oil seeds, processed to low moisture content and so fragmented as to permit dilution with water, milk or other suitable medium; and should contain minimum 75% cereals/legumes (excluding added sucrose). Several types of weaning foods are available in India but they are milk based and expensive such as Cerelac, Nestle. Although, few cereal based weaning foods such as Maruti, Continental Milkose (India) Ltd. have been recently introduced in Indian market, but still, not popular as much as Cerelac. The advantage of developing cereal-based weaning foods is to enhance nutritional value in terms of micronutrients at a lower cost. Additionally, cereals could act as better base material for fortification of iron and vitamin A as compared to milk. On the contrary, milk and milk products pose various organoleptic challenges such as adverse color and flavor develops frequently during the processing and storage. Therefore, the present investigation was designed to develop a cereal-based iron and vitamin A fortified weaning food and evaluates its nutritional and physicochemical attributes.

MATERIALS AND METHODS

Weaning food ingredients and packaging material: Good quality pearl millet (Pennisetum glaucum) grains (Pro-Agro's 9444) were procured from pearl millet breeding farm, Haryana Agriculture University, Hisar. Wheat grains (PBW-343) were procured from a reputed merchant located in local market of Jind, (Haryana). Skim milk powder (SMP) was purchased from M/s Modern Dairies Ltd., Karnal (Haryana). Whey protein concentrate-70 (WPC-70) containing 70% protein was purchased from M/s Modern Dairies Ltd., Karnal (Haryana). Sucrose-Extra-pure was used as the source of sugar. L-Ascorbic Acid (Vitamin C, food grade) from Sisco Research Laboratories Pvt. Ltd., Mumbai was added in the molar ration of 4:1 (ascorbic acid: iron) to enhance the bioavailability of the fortified iron salt. Aluminium foil laminates (thickness 100µm) were procured from local market. All the chemicals used in the preparation of different reagents were of analytical grade and were procured from reputed companies. The reagents required for analysis were freshly prepared adopting standard procedures. Electrolytic iron was procured from Sisco Research Laboratories Pvt. Ltd., Mumbai and Hydrogen reduced iron from Sigma-Aldrich, St. Louis, MO, USA. Retinol acetate (vitamin A acetate) was procured from DSM Nutritional Products India Pvt. Ltd, Mumbai, India.

Pearl millet grains were washed and soaked in tap water for 9 h at 30°C followed by controlled germination for 40h at 25°C and 80% relative humidity. The germinated grains were washed again under tap water and then dried in fluidized bed dryer at 90°C for 75 min and rootlets were removed manually. Dehulled and dried grains were then pearled and then moisture level was adjusted to 13%. Grains were then extruded in single screw extruder followed by milling. Wheat grains were washed in tap water, moisture level adjusted to 12% and extruded in single screw extruder followed by milling with a fine sieve attached to the flour mill. Various combinations of the product using pearl millet flour, wheat flour, sucrose, SMP and WPC-70 were prepared (Table 1) and optimized on the basis of sensory acceptability. Thus, the optimized formulation was further fortified using electrolytic iron (6, 9, 12 mg/100g), hydrogen reduced iron (6, 9, 12 mg/100g) and vitamin A acetate (300, 330 and 360 µg /100g) for further sensory evaluation. The molar ratio of ascorbic acid to iron was kept 4:1 in all the combinations. The final product was vacuum packaged in aluminium foil laminates of thickness 100µm at 25°C for storage.

Nutritional analysis: The moisture content, crude fat, crude protein, crude fiber and ash content were estimated as per the standard methods of AOAC (2005). Iron content was estimated using atomic absorption spectrophotometer as per the standard of AOAC (2005) method using dry digestion. ß-carotene extraction, saponification and estimation was carried out using method of Hawe and Sherry (2006).

Estimation of vitamin A content: Vitamin A content was extracted and estimated as per the method of Kazmi et al. (2007). Vitamin A was measured and reported as International Unit/liter (IU/L). One IU of vitamin A acetate or palmitate is equivalent to 0.33µg vitamin A. For the preparation of vitamin A standard, 13.2µg vitamin A acetate was accurately weighed and transferred into 10ml low actinic volumetric flask and dissolved in methanol and volume was made up to 10ml. This stock solution had vitamin A concentration of 435600 IU/L and was diluted to such an extent that the resulting solutions were having concentration of 100, 1000, 2000, 3000 and 4000 IU/L.

Physicochemical analysis: Water absorption index of the developed product was determined as per the method of Anderson et al. (1982). To determine water solubility index,
10g of sample was dispersed slowly in 100ml of distilled water (65°C) with continuous stirring to dissolve it completely. Prepared solution (50 ml) was transferred in centrifuge tubes. The tube was centrifuged for 5 min at 3000 rpm and the supernatant was decanted. Again distilled water (65°C) was added in tube to make up the volume to 50ml and centrifuged again for 5 min. The supernatant was finally decanted and volume of the solid remained in tube was noted. The solubility index was expressed as per cent. Flowability of the product was determined in terms of ‘angle of repose’ as per the method given by Sjollema (1963).

Water Activity (a_w): Water activity (a_w) of developed product was measured by using water activity meter. For determination of a_w, 2g of representative sample were weighted in a plastic dish, supplied with instrument, and subjected to instrumental measurement.

Colour (L*, a*, b* values): The colour of the developed product was measured using a colourflex provided with the universal software (Version S.10). Before the test, the instrument was calibrated with standard black glass and white tiles as specified by the manufacturer. The light source was dual beam Xenon flash lamp. Colour values were expressed in terms of CIE-(L*), (a*), (b*) uniform colour specs, where (L*) indicates lightness, ranges zero (black) to 100 (white), (a*) indicates redness (0 to +60) and greenness (0 to –60) and (b*) is the amount of yellowness (0 to +60) and blueness (0 to –60). The samples were filled densely up to 1cm height in the sample container.

Microbiological analysis: Well-mixed sample (11g) was transferred to a dilution bottle containing 99ml sterile saline solution and mixed gently by shaking to prepare 1:10 dilution. The 1:10 dilution thus obtained was used for making further dilutions, for subsequent bacteriological analysis. Standard plate count was determined on standard plate count agar as described by Marshall (1993). Coliform counts as well as yeast and mold counts were recorded by as per the method given by Marshall (1993).

Sensory evaluation: Sensory evaluation of the weaning food was carried out using 9 point Hedonic scale. Samples were evaluated for colour, taste, appearance and overall acceptability. The sensory evaluation of the weaning food was performed with an evaluation panel of 10 semi-trained members.

Cost calculation of developed product: After standardizing the ingredients, processing parameters and process control for manufacturing weaning food, the cost of production was estimated according to the method suggested by Patel (2012).

Statistical analysis: All the data (Except sensory data) was calculated in triplicates using Microsoft Excel 2010 (Microsoft Corp., Redmond, WA) and presented as mean ± SEM. Sensory data is calculated for n=30 and shown as mean ± SEM.

RESULTS AND DISCUSSION

Various combinations of pearl millet flour, wheat flour, SMP, WPC-70 and sucrose were evaluated for sensory acceptability. Results indicated that T1 combination was most preferred (Sensory data not shown), which was further fortified using iron salt, vitamin A acetate and ascorbic acid.

Effect of iron fortification on sensory acceptability of weaning food: Technically, iron is the most challenging micronutrient to add to foods, because the iron compounds that have the best bioavailability tend to be those that interact most strongly with food constituents to produce undesirable organoleptic changes. FSSR (2010) guidelines permits only the elemental iron salts (electrolytic iron or hydrogen reduced iron) for fortification of processed cereal-based weaning foods. Elemental iron powders are the fortificants used most widely in wheat flour and other cereal products, including infant foods, because the cost of iron in this form is lower and it is not likely to affect the storage properties and shelf life of fortified flour, or the colour and taste of the final food products (Hurrell et al. 2002). Recommended Dietary Allowances (RDA) of iron for Indian children between the age of 1 to 3 year is 9mg (ICMR 2009a). The native iron content in the unfortified developed cereal-based weaning food was 3mg per 100g, thereby the product was deficient by 6mg of the iron required to meet the RDA for Indian children. At the same time it is said that the bioavailability of electrolytic iron is approximately half than the ferrous sulphate (Hurrell et al. 2002), hence the minimum level of iron salts required to fulfill the 100% RDA is 12mg per 100g of the product. FSSR (2010) also states that the fortified products should meet minimum 40% of the RDA through any single fortified product. Therefore, in the present investigation, three concentrations (6, 9 and 12mg) of the iron salts were studied. Vitamin C can increase the absorption of both native iron and fortified iron several fold when added to foods. It reduces ferric form to ferrous ion and prevents or decreases the formation of insoluble complexes with absorption inhibitors or with hydroxide ions in gut (Hurrell et al. 2002). Ascorbic acid to iron molar ratio of 4:1 is adequate for foods having more phytate content (Lynch et al. 2009).

It is evident from the Table 2 that there was no significant difference in sensory scores of any of the parameters, on addition of electrolytic iron at the level of 12mg per 100g of the product. However, in case of hydrogen reduced iron, the addition of 6mg of the said iron salt per 100g of the product affected the flavour, colour and appearance and overall acceptability to a statistically significant (P<0.05) level. Hence, the electrolytic iron was selected to fortify the developed cereal-based weaning food. Keeping the bioavailability and RDA in mind, 12 mg electrolytic iron salt per 100g of the product concentration was finalized.

Effect of vitamin A fortification on sensory acceptability of weaning food: Vitamin A is an essential nutrient that is required in small amounts by humans for the normal functioning of the visual system, the maintenance of cell function for growth, epithelial cellular integrity, immune function and reproduction. Vitamin A deficiency is among top-ten health problems contributing to the global burden of disease. Therefore, Government of India recommends that
the child should be given vitamin A supplements every six months from nine months to three years to avoid vitamin A deficiency (Arnold et al. 2009). Fortified complementary foods could be effective in preventing and controlling vitamin A and other common nutritional deficiencies in infants.

The recommended dietary allowance (RDA) of vitamin A for Indian children between age of 1 to 3 years is 400µg (ICMR 2009b). FSSR (2010) guidelines also states that the fortified products should meet minimum 40% of the RDA through any single fortified product. Vitamin A acetate and vitamin A palmitate has been permitted by FSSR (2010) and either of the two can be used in the fortification of cereal-based weaning foods. The unfortified cereal-based weaning food developed during the present investigation had 33µg per 100g of native vitamin A, thereby the product was deficient by approximately 367µg of the vitamin A required to meet the RDA for Indian children. In the present study, vitamin A acetate was further fortified in developed weaning food, which was already optimized in reference to iron concentration (i.e. 9mg/100g) (Table 3). Sensory data revealed that iron fortified product showed non-significant scores at all the levels of vitamin A studied. It can be calculated that the developed weaning food fortified with 360µg/100g was able to meet 90% of RDA of vitamin A for children between ages of 1 to 3 years without affecting the sensory characteristics of the developed product. Similar results have been reported by Sihag et al. (2015), who fortified vitamin A in cereal-based weaning food and found the product palatable up to 6 and 4 months when stored under vacuum and normal atmospheric conditions, respectively. Hence, it may be concluded that the iron and vitamin A fortified weaning food developed in the present investigation has the ability to meet the 100% of RDA for iron and 98.25% for vitamin A.

**Nutritional characteristics of the developed weaning food:** Literature suggests that pearl millet is equal or even superior in protein content as compared to wheat, rice, maize and sorghum grains (FAO 2005). Since pearl millet form good sources of macro- and micro-nutrients, these millet grains could serve as promising staple grain for therapeutic diets (Chandrasekara and Shahidi 2013). Nambiar et al. (2011) also described comprehensively the possible health benefits of pearl millet in reducing the risk of diabetes, cancer, anaemia and constipation, etc. The developed processed pearl millet based weaning food with optimized levels of iron, salt and vitamin A acetate was analyzed for nutritional composition. The average moisture content of the developed weaning food was 5.73±0.02% and average protein, fat, carbohydrate, ash and crude fibre content (on dry weight basis) was 15.32±0.06, 1.96±0.07, 79.58±0.06, 2.02±0.02 and 1.12±0.10%, respectively. The iron, vitamin A and ß-Carotene content were found to be 15.0mg/100g, 393µg and 30.62µg per 100g of the product. Almeida-Dominguez et al. (1993) evaluated

<table>
<thead>
<tr>
<th>Sensory attributes</th>
<th>Unfortified product Control</th>
<th>Iron fortified product (per 100g)</th>
<th>Hydrogen reduced iron</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Electrolytic iron</td>
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<tr>
<td></td>
<td></td>
<td>Control +6mg</td>
<td>Control +9mg</td>
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<tr>
<td></td>
<td></td>
<td>Control +12mg</td>
<td>Control +12mg</td>
</tr>
<tr>
<td>Colour and appearance</td>
<td>8.01±0.11a</td>
<td>7.95±0.05a</td>
<td>7.92±0.03a</td>
</tr>
<tr>
<td>Flavour consistency</td>
<td>8.26±0.08a</td>
<td>8.20±0.05a</td>
<td>8.15±0.05a</td>
</tr>
<tr>
<td>Consistency</td>
<td>7.87±0.05a</td>
<td>7.79±0.06ab</td>
<td>7.75±0.06ab</td>
</tr>
<tr>
<td>Overall acceptability</td>
<td>8.24±0.07a</td>
<td>8.16±0.13a</td>
<td>8.10±0.08a</td>
</tr>
</tbody>
</table>

Data are presented as means ± SEM (n=30). Means within rows with different superscript are significantly different (P<0.05) from each other.

<table>
<thead>
<tr>
<th>Attributes</th>
<th>Iron fortified product Control</th>
<th>Fortified product [with vitamin A acetate (µg /100g)]</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Control +300*</td>
<td>Control +330*</td>
</tr>
<tr>
<td>Colour and appearance</td>
<td>8.02±0.10a</td>
<td>7.96±0.09a</td>
</tr>
<tr>
<td>Flavour</td>
<td>8.28±0.11a</td>
<td>8.21±0.10a</td>
</tr>
<tr>
<td>Consistency</td>
<td>7.80±0.11a</td>
<td>7.81±0.18a</td>
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<tr>
<td>Overall acceptability</td>
<td>8.22±0.14a</td>
<td>8.18±0.10a</td>
</tr>
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*indicates the concentration of vitamin A acetate in µg /100g. Data are presented as means ± SEM (n=30). Means within rows with different superscript are significantly different (P<0.05) from each other. 6Control means weaning food fortified with 9 mg electrolytic iron/100g of the product as optimized on the basis of Table 2.
the nutritional quality of weaning food blend (70% pearl millet and 30% cowpeas), which possessed 3.9% moisture, 17.9 g protein, 3.2% fat, 72.9% carbohydrates and 1.6% ash. Zaheeruddin (2011) has reported the similar results for weaning food prepared from extruded pearl millet, barley and SMP, the reported values for proximate composition i.e. protein, fat, carbohydrate, crude fibre and ash being 15.29, 1.11, 73.47, 1.03 and 2.79%, respectively. Sumathi et al. (2007) formulated pearl millet based (85% pearl millet and 15% Soya) extruded supplementary food, which possessed 16% protein, 4.4% fat, 61.3% carbohydrates and 1.7% dietary fibres.

Physical properties of the developed weaning food: Physical properties of the developed weaning food namely flow-ability, water absorption index (WAI), water solubility index (WSI) and colour were analysed. Flowability is a significant characteristic of powdered materials as it affects the handling as well as its movement through channels during the manufacturing and packaging at industrial scale. Product with poor flowability might need more energy and may clog the pipes during handling. Flowability can be indicated in terms of angle of repose. Carr (1965) suggested that the formulations with angle of repose in the range of 31–35 have good flowability. Angle of repose lower than 31 indicates excellent and more than 35 indicates poor flowability. In the present study, the angle of repose of the product was found to be 33.80±0.27. Hence, the result indicated that the product was quite flow-able. The water solubility index and water absorption index measures the volume occupied by the product after swelling in excess of water, which maintains the starch in aqueous dispersion. A high WSI indicated that starch underwent extensive conversion during the processing such as extrusion. It also describes the rate and extent to which the component of powder material or particles dissolves in water. WSI and WAI in the fortified developed weaning food were 9.21±0.52% and WAI 3.96±0.04 – 6.87±0.03% of the extrusion process and reported low WSI (5.67±0.08 – 7.26±0.10%) respectively. Similarly, Sawant et al. (2013) prepared ready-to-eat food from finger millet based composite mixer by extrusion process and reported low WSI (5.67±0.08 – 9.21±0.52%) as well as WAI (3.96±0.04 – 6.87±0.03%) of the product. However, Balasubramanian et al. (2011), who worked on the optimization of weaning mix based on malted and extruded pearl millet and barley, reported very high WSI (39.65–47.25%) and very low WAI (2.30–2.91%). In the present study, lower WSI of the product could be attributed to higher quantity of cereals flour and thus, more gelatinization of starch (Rasane et al. 2013). The results are in accordance with the findings of Rasane et al. (2015), who developed fermented baby food by employing pearl millet, sorghum and oat flour and found good solubility index of the product.

Water activity (a_w) of the food product is an important determinant of various deteriorative reactions and thus their shelf-life. Water activity (a_w) refers to amount of free water that is available in food for microbial growth and is an important means of predicting and controlling the shelf life of food products. It may be influenced by its formulation, composition and it also affects the reconstitution properties. Lower water activity value indicates enhanced stability of the product during storage from microbial point of view. The water activity of the developed cereal-based weaning food was found to be 0.29±16 at 25°C. Colour is an important quality factor directly related to the acceptability of food products and is an important physical property to report for extruded products. Colour changes can give information about the extent of browning reactions such as caramelization, maillard reaction, angle of cooking and pigment degradation during the extrusion process (Ilo and Berghofer 1999). The lightness (L*), redness (a*), yellowness (b*) for the developed weaning food were 77.87±0.26, 2.15±0.12 and 17.43±0.08, respectively. The lightness (L*) or brightness of the product affects the consumer acceptability and is desirable for the weaning foods (Almeida-Dominguez 1993). Due to dominance of milk-based weaning foods in market, the consumers’ perception is already towards light coloured baby foods. Therefore, during the formulation of any new food product, utmost care should be taken while selecting the ingredients, so the overall acceptability of the product is not affected negatively. This value in the present study is towards the higher side (77.87) because of the combined effect of wheat flour, SMP and WPC-70 content of pearl millet flour. Lower a* value (2.15) of the product indicates lower content of maillard reaction products formed during the extrusion process. The yellowness value (b*) indicates extent of non-enzymatic browning during the processing; (b*) value of the developed weaning food was found to be 17.43, which may be due to the effect of non-enzymatic reactions and pigment destruction reactions during extrusion cooking (Guy 2001). Sawant et al. (2013) developed few combinations of snack food using finger millet, soy, maize, bengal gram and rice flour only by employing extrusion technology and reported lightness (L*) in the range of 31.39–45.79. However, they obtained the similar values in case of a* and b*. In the present study, higher L* of prepared weaning food could be attributed to the presence of wheat flour, sucrose and milk solids.

Microbiological analysis: Coliforms, yeast and mold were absent and standard plate count was too few to count, which could be attributed to the lower moisture content and water activity of the prepared product. Other reason for it could be the vacuum packaging which could protect the product from contamination as well as growth of...
microorganisms during storage. Similarly, Rasane et al. (2014) developed baby foods based on pearl millet, oats and sorghum and reported that the product was microbiologically stable with no coliform and organisms such as Clostridium botulinum, Salmonella spp. and Staphylococcus aureus detected.

Cost of the developed product: The cost of production of the processed pearl millet based weaning food was estimated as ₹ 193.40/kg. Since the commercially available milk based weaning food is sold in 350g laminated pouches, hence the cost of the developed product is ₹ 67.70/pouch of 350g. Under commercial conditions at higher scale of handling, the cost of production is expected to be lower. The present cost of the commercially available milk based weaning food (Cerealac) in Indian market is ₹ 137/350g.

It can be concluded from the study that pearl millet can be a good alternative for the formulation of micronutrients enriched weaning food, which could be formulated at a lower cost with higher palatability and better nutritional attributes. The developed product was successfully fortified with iron and vitamin A and could meet the RDA as suggested by Indian Council of Medical Research, India. The developed weaning food is ready-to-eat with desirable flowability, water absorption index, water solubility index and colour characteristics. Results also indicated that electrolytic iron is a better choice over the hydrogen reduced iron for the fortification of cereal-based weaning foods, as former did not affect the colour and sensory acceptability of the final product negatively.

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