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FERMENTATION OF SOYMILK BY COMMERCIAL LACTIC CULTURES: DEVELOPMENT OF A PRODUCT WITH MARKET POTENTIAL

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Soymilk supplemented with 2% sucrose was inoculated with a mixture of *Streptococcus thermophilus*, *Bifidobacterium lactis* and *Lactobacillus acidophilus*. The fermentation was monitored by pH as a function of time. This sample was used as a basis for the formulation of beverages flavoured with pineapple, strawberry, coconut, kiwi, guava and hazelnut. The beverages were submitted to a sensory acceptance test with consumers using the nine-point structured hedonic scale. ANOVA and Preference Mapping were used to analyse data and results showed higher significant ($P<0.05$) acceptance for pineapple and guava flavours. The strawberry, kiwi and coconut flavours obtained acceptance close to 6.0 (liked slightly), while the hazelnut flavour was rejected (acceptance less than 5.0). This study demonstrated that it is possible to formulate highly acceptable soymilk beverages by way of lactic fermentation and addition of flavourings.

Keywords: soymilk, fermentation, sensory evaluation, preference mapping

Recently, the Brazilian market for beverages has been expanding significantly with the introduction of new markets aimed at a consumer segment in search of low calorie foods with high nutritive value and a natural, healthy appeal. Of these products, the following stand out: ready to serve fruit juices, isotonic beverages and lactic beverages, including flavoured milks, chocolate flavoured products and milks fermented by probiotic micro-organisms. This new segment of products, known as health drinks, represents a world-wide tendency, and its market is mainly formed by well educated, well-off consumers, i.e., individuals more conscious of the importance of a healthy diet and concerned about consuming foods which bring some health benefits (BEHRENS et al., 2000).

Of the products of vegetable origin with a good potential for developing new beverages, the water-soluble soybean extract stands out. Popularly known as "soymilk", this product was formerly rejected by Brazilian consumers on account of its characteristic flavour, which is similar to raw beans. This disagreeable aspect of the flavour is caused by aldehydes, especially pentanal and *n*-hexanal, which are formed by enzymatic oxidation of the lipids present in the soybean (PATEL et al., 1980).

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The interest in using soybean for human consumption arises from its chemical and nutritional characteristics, which qualify it as a functional food, i.e., a food which, in addition to its nutritional function, possesses substances which help diminish the risk of certain diseases. To complement this, studies have shown that the soybean can be used for preventative and therapeutic purposes in the treatment of cardiovascular diseases, cancer, osteoporosis and menopause symptoms (HASLER, 1998).

On the other hand, it is not enough for a food to have good nutritional characteristics and good health allegations to be well accepted by the consumer, since in addition to making his choice based on information provided on the label, the consumer will also evaluate price, convenience for use and preparation, and above all, the sensory quality of the food. Thus a food such as soymilk, with a low sensory consumer acceptance by western populations, needs to have its sensory characteristics, notably its aroma and flavour, modified to become more attractive to non-Asiatic individuals.

From the technological point of view, one of the ways of improving the sensory quality of soymilk is by lactic fermentation. The fermentation of the water-soluble extract of the soybean by lactic cultures has been extensively studied in the last two decades. The low level of fermentable sugars in soybean has limited the use of soymilk as a substrate for the growth of lactic cultures such as *S. thermophilus, L. bulgaricus, L. acidophilus* and *L. casei.* However, studies have shown that supplementation with sucrose, glucose and lactose has made soymilk a suitable substrate for fermentation by lactic bacteria, thus obtaining products with appreciated sensory properties (WANG et al., 1973). From the sensory point of view, the fermentation of soymilk by *L. acidophilus* and/or *L. casei* contributed to an improvement not only in the flavour of the product but also in the aroma. The proteolytic activity of these organisms, especially that of *L. casei*, is responsible for the formation of flavour and aroma precursors, contributing to the elimination of the off-flavour caused by *n*-hexanal (MURTI et al., 1993).

As a result, the fermentation of the water-soluble soybean extract by probiotic micro-organisms could create a product with excellent market potential, both for its health appeal and for the sensorial quality added to the product by these organisms.

Thus, with the objective of developing a fermented beverage based on the water-soluble soybean extract, this research focused on the following: i) monitoring of the fermentative process of soymilk, testing three different lactic cultures, using sucrose and lactose as growth nutrients, and by way of sensory consumer tests, defining the best fermentation conditions for the basis of the new product, ii) development of the new fermented soymilk beverage with different flavours, using consumer tests and Internal Preference Mapping to evaluate the acceptance of the formulations.

1. Materials and methods

1.1. Materials

Water-soluble soymilk extract was used as the substrate for lactic fermentation. To ferment the product, a commercial lactic culture was used, BIO-RICH® Probiotic (CHR Hansen A/S, Hørsholm, Denmark), which consisted of *Streptococcus thermophilus,* combined with *Lactobacillus acidophilus* and *Bifidobacterium lactis*.

1.2. Fermentation

Due to the low carbohydrate content of the raw material, 2% sucrose was added to serve as the growth nutrient for the lactic bacteria. The concentration of 2% of each carbohydrate was determined based on data in the literature for the fermentation of soymilk (MITAL & STEINKRAUS, 1974; PATEL et al., 1980).

Fermentation was monitored by pH measurements every hour and continued until the pH was in the range between 4.0 and 4.5. This range was stipulated for both preservation and sensory reasons.

1.3. Formulation of beverages based on the fermented soymilk

Six different formulations of a "ready to drink" product similar to yoghurt were prepared with different flavours. Table 1 shows the composition of each formulation.

Table 1. Composition of the beverages based on the fermented soymilk, flavoured with six different flavours

Flavour	Amount fermented soybean milk (%)	Amount sucrose (%)	Flavour[a]	Dye[b]
Pineapple	90	10	pineapple essence BRO18343-0	tartrazine yellow plus twilight yellow
Coconut	90	10	coconut essence BRO18315-4	No dye
Strawberry	90	10	strawberry with cream essence T210894 and strawberry essence BRO18306-5	artificial red dye VI
Kiwi	90	10	kiwi essence BRO18327-8	mixture yellow V, blue I and blue II
Hazelnut/ Vanilla	90	10	hazelnut essence T210698 and vanilla essence T210699	caramel – ammonia process type C
Guava	90	10	guava essence BRO18315-4	artificial red VI tartrazine and twilight yellows

[a] Flavours provided by IFF – International Flavour and Fragrance, Taubaté, SP, Brazil.
[b] Dyes brand Arcolor® – Arco-Iris do Brasil Ind. Com. De Prod. Alim. Ltda., São Paulo, SP, Brazil.

1.4. Sensory acceptance of the formulated beverages

An affective test was carried out with the six samples described in Table 1 with the objective of determining the degree of acceptance of each formulation and how ideal were the sweetness and characteristic flavour intensities, according to potential consumers of the product.

The samples were prepared on the eve of the test and placed in plastic bottles (PET) under refrigeration.

Ninety-four consumers participated in the test, all recruited from the Campinas State University campus and were between 18 and 50 years of age, 50% being male and 50% female.

Aiming at minimising the first order effect in the sample evaluation, a vanilla-flavoured "dummy" sample was always served at the first position. The values awarded to this sample by the consumers were discarded. After judging the "dummy" sample, panellists were served the six formulated beverage in a single session with a monadic presentation, the order being determined by the experimental design proposed by WAKELING and MACFIE (1995) consisting of complete balanced and randomised blocks. Four sensory attributes were evaluated using the nine-point hedonic scale: appearance, aroma, flavour and overall liking. Sweetness and flavour intensity were evaluated by means of Just-about-right (JAR) scale (STONE & SIDEL, 1993). During the test, panellists were asked to rinse their mouths with water between samples to avoid sensory fatigue.

The acceptance test was carried out in individual booths in the Sensory Analysis Laboratory of the Faculty of Food Engineering at the State University of Campinas, Brazil.

The data referring to the acceptance of the samples were submitted to a two-way ANOVA (samples and consumers) followed by the Tukey means comparison test. Data evaluation was performed using the "Statistical Analysis Systems" programme (SAS, 1992).

Overall liking data were analysed by the Internal Preference Mapping methodology, too – MDPREF (MACFIE & THOMSON, 1988; MACFIE et al., 1996), which allowed for the creation of a tri-dimensional affective sensory space comprising the 94 consumers and the six samples.

2. Results and discussion

2.1. Fermentative process of the soymilk

The stability, aroma, flavour and texture of fermented soymilk products depend on the pH. According to OBERMAN (1985), the optimum pH for products of this type is between 4.2 and 4.5. Below pH 4.0, the product becomes too acidic and the soybean proteins precipitate. On the other hand, pH values above pH 4.5 compromise the conservation of the product and also its flavour, since the improvement in sensory

quality of soymilk is obtained by the masking of volatile soybean compounds such as *n*-hexanal and pentanal by fermentation products, especially compounds such as lactic acid, acetaldehyde and diacetyl. Thus in this study, the fermentative process was monitored until the sample pH reached this range.

In a previous affective sensory test, samples of soymilk fermented by the BIO-RICH® Probiotic culture presented, according to the consumers, milder notes of aroma and acidity characteristic of yoghurt and other aroma notes which reminded them of honey, flowers and sweet things, that is, of agreeable aromatic notes (BEHRENS et al., 2000). On the other hand, fermentative process of the soymilk by the lactic culture BIO-RICH® Probiotic took only 5 h to reach the desired pH value (near to 4.5). Thus, due to its short fermentation time and the sensory quality of the product, BIO-RICH® Probiotic culture was shown to be appropriate for industrial fermentative process of soymilk.

2.2. Formulation of the fermented soymilk based beverages: consumer test

Table 2 shows the acceptance mean scores of appearance, overall liking, aroma and flavour of the formulated beverages based on fermented soymilk flavoured with six different flavours: pineapple, strawberry, kiwi, hazelnut, coconut and guava. These results correspond to the responses obtained with 94 potential consumers of the product developed.

With respect to appearance, all samples obtained mean score of about 7 on the hedonic scale, corresponding to the term "like moderately", the pineapple and guava flavoured samples standing out from the rest, with means for appearance of 7.5 and 7.7, respectively.

In the overall liking evaluation, the pineapple and guava flavoured samples were again the most accepted, obtaining mean scores close to 7, corresponding to "like moderately" on the hedonic scale. These values indicate that these two samples were well accepted by the consumers, presenting high market potential. The kiwi, strawberry and coconut flavoured samples obtained mean score for overall acceptance of about 6 on the hedonic scale ("like slightly"), and the hazelnut flavoured sample was rejected by the judges, receiving a mean score of 4.9 (below the category "neither like, nor dislike" on the hedonic scale).

With respect to sample aroma, it was shown that the acceptance means were between 5 and 7, that is, between the categories "neither like nor dislike" and "like moderately" on the hedonic scale. The pineapple flavoured sample obtained the highest mean (6.9), whilst the hazelnut sample received the lowest mean (5.4). The remaining four samples received similar means for aroma.

The pineapple and guava flavoured samples again obtained the highest acceptance mean scores of about 7. The coconut, kiwi and strawberry flavoured samples obtained similar means for flavour of about six, whilst the hazelnut samples were rejected by the consumers, with a mean acceptance for flavour below 5.0 on the hedonic scale, that is, below the region of product approval.

Table 2. Acceptance mean scores for the sensory attributes of the fermented soymilk based beverages (1: dislike extremely, 5: neither like, nor dislike, 9: dislike extremely)

Beverage	Appearance	Overall liking	Aroma	Flavour
Pineapple	7.5a	7.1a	6.9a	7.0a
Coconut	6.7d	6.2c	6.3ab	6.0bc
Strawberry	7.4abc	6.4bc	6.3ab	6.0c
Kiwi	6.9cd	6.2c	6.3ab	6.0c
Hazelnut	7.0bcd	4.9d	5.5c	4.8d
Guava	7.7a	7.0a	6.2b	6.7ab

Means with the same letter do not differ at the 5% level of significance.

Most consumers considered the sweetness of the six samples to be ideal, especially with respect to the pineapple flavoured sample, which was considered ideal for sweetness by 75% of the consumers. This fact indicated that the higher or lower sample acceptance (Table 2) was not due to the amount of sugar used in the formulations.

With respect to the flavour intensity, results showed that most consumers considered the intensities of each flavour close to the ideal point of the JAR scale. Considering the concentrations of flavouring used in the formulations, which followed the suggestions of the manufacturers, it was concluded that the pineapple produced the best flavour impact, producing a beverage highly accepted by the consumers.

2.3. Internal Preference Mapping (MDPREF)

Figures 1 and 2 present the Internal Preference Map generated by the values for global acceptance of the samples by the consumers. The 94 individual consumer responses generated a multidimensional sensory space represented by dimensions, which explain the total variation of the consumer responses. In this study, the first three preference dimensions explained about 71% of the total variability of the consumer responses in overall liking of the six beverages, each being represented by a square in the affective sensory space in Figs 1 and 2. Consumers were represented by lozenges located closer to the preferred beverages and distant from those less accepted.

The MDPREF showed that 76% of the consumers (lozenges) can be found close to the pineapple, strawberry and guava flavoured samples, indicating that these were the samples individually preferred by the great majority of the consumers. The coconut and kiwi flavoured samples, located in the central region of the MDPREF, represent a segment of intermediate acceptance, and the hazelnut flavoured samples, situated to the left, represent the least preferred samples, that is, the least accepted samples by the consumers. However, a segment of the consumers did prefer the hazelnut sample, and these were located closer to this sample on the MDPREF, representing a total of only 6% of the individuals.

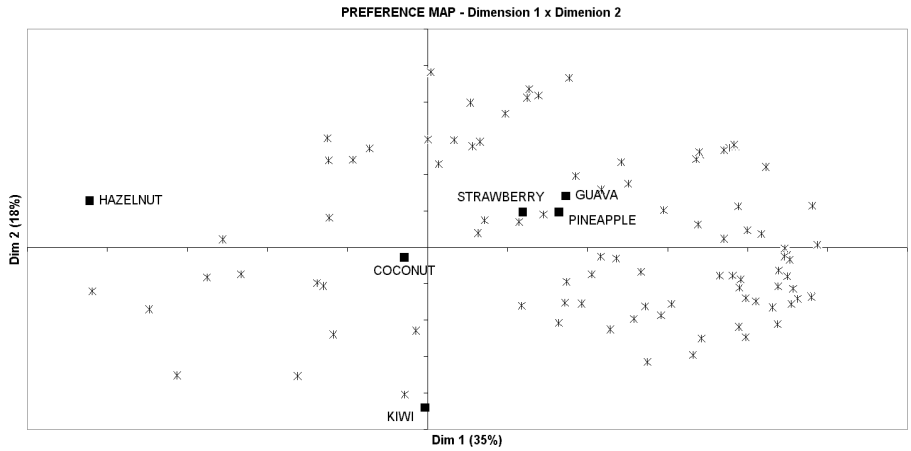

Fig. 1. Internal Preference Map, representing the first and second preference dimensions, obtained from the acceptance scores of the six samples of flavoured beverages formulated with fermented soymilk (represented by named squares). Consumers are represented by lozenges

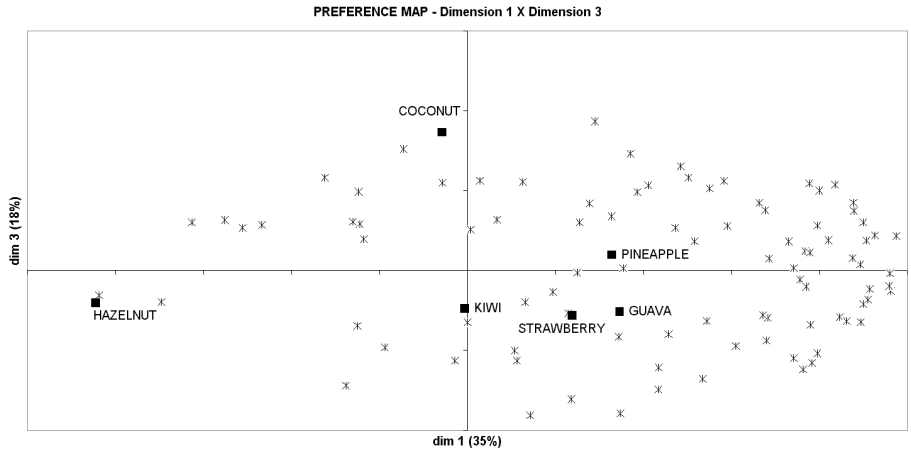

Fig. 2. Internal Preference Map representing the first and third preference dimensions, obtained from the acceptance scores of the six samples of flavoured beverages formulated with fermented soymilk (represented by named squares). Consumers are represented by lozenges

The conclusions obtained from the MDPREF confirmed and complemented the overall liking data listed in Table 2, showing that the pineapple and guava flavoured samples really were preferred by the majority of the consumers (76%), followed by the

strawberry flavoured samples. The kiwi and coconut flavoured samples obtained very similar overall liking scores, and represent a group of intermediary preference. The hazelnut flavoured sample was the least appreciated by the consumers in general, only a very small segment preferring this sample (6%).

The technique of Internal Preference Mapping (MDPREF) was shown to be an efficient tool in the development cycle of new products, since it allows for the observation of individual preference segmentation of the consumers as a function of the sensory characteristics of the evaluated products. Thus, products that better meet consumer expectations and therefore have greater success potential in their segment of the market can be chosen with greater assurance.

3. Conclusions

Starting from the soymilk with 2% added sucrose and fermented by the BIO-RICH® Probiotic culture – appropriate for a new product development, both with respect to the fermentation time of only 5 h and the sensory quality of the final product – it was possible to formulate ready to drink, yoghurt-like beverage of good consumer acceptance. With pineapple and guava flavours, this product showed higher acceptance by the consumers for all the attributes evaluated, thus showing good market potential. When the traditional flavours of strawberry and coconut and the new flavour of kiwi were used, the products also obtained good acceptance by the consumers, although to a lesser degree. The hazelnut flavour – uncommon in dairy products in Brazil – was rejected by local consumers. Thus, the results showed the potential of soymilk fermentation by a commercial lactic culture in the development of new products of good sensory quality and high consumer acceptance. In addition to its sensory qualities, the nutritional and health claims of this new soymilk beverage could also be used in the promotion, increasing its market appeal.

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