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APPLICATION OF lactium™ AND RECOVERY OF THE ANTI-STRESS PEPTIDE αS1-CASEIN (f91-100) IN YOGHURT AND BEVERAGES

SUMMARY

The addition of proteins and/or peptides to drinks often introduces instability phenomena in drinks affecting appearance and/or mouthfeel. NIZO food research helped us in using its pilot plant facilities and expertise on stability of drinks, to identify and test new beverage application areas for Ingredia. The aim of this study was among many to evaluate the impact of lactium™ on taste, texture and recovery in a broad spectrum of beverages, notably fruit drink, and to compare the beverages to a reference without lactium™.

In this project, the beverage concept "mango fruit drink" was tested. Common industrial ingredients were used to successful stabilise lactium™. The addition of lactium™ did not significantly affect the taste of the drink.. The mango fruit drink showed minor visual changes.

In order to determine the recovery of αS1-casein-(91-100)-peptide in beverages, a new sample preparation method for reverse-phase HPLC was developed. This method was used to analyse the effect of processing and storage on αS1-casein-(91-100)-peptide in the drink. The recovery was not affected in fruit drink.

1. INTRODUCTION

The applied concentration of lactium™ in the beverage was 0,6 g/L for the fruit drink (250 g serving size). The products was texturally and chemically characterised and sensory evaluated and compared to a blank (without added lactium™). In order to determine the recovery in the drink a new method was developed.

2. MATERIALS & METHODS

2.1. Production and evaluation of drinks

The ingredients and production of model drinks was based on industrially relevant formulations and processing, which were discussed with Ingredia prior to the production.

2.1.1. Production of model drinks

- Fruit drink *light*

The fruit drink production is schematically given in Figure 3 and was based on mixing ingredients, homogenisation and pasteurisation. A more detailed description is given below.

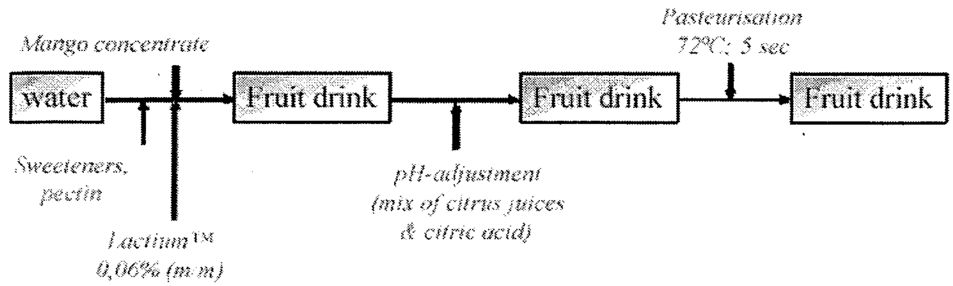

Figure 3 Flow chart of mango fruit drink production.

The mango fruit drink was prepared adding a dry mix of Grindsted® pectin RS4613 (Danisco, Czech Republic) and a mixture of artificial sweeteners (cyclamate, saccharine and acetosulfam-K) (Natrena®, The Netherlands) to water until a clear solution was obtained. The amount of sweetener added was equivalent to 4,5% sucrose (m/m). Finally concentrated fruit drink was added. The pH was controlled and adjusted to pH 3,5 and the formulated mango fruit drink (Table 3) was prepared using the Combitherm (NIZO food research) by successively pre-heating (70°C), homogenisation (200/40 bar), heating (5 sec at 72°C), cooling and filling into sterile 0,5 L beakers.

Table 3 Formulation of concept mango fruit drink. Serving size 250 ml.

Ingredients	Blank (% m/m)	With lactium™ (% m/m)
Water	85	85
Mango concentrated juice (>70%)	14	14
Sweetener (mixture cyclamate, saccharine, acetosulfaam-K)	<0,1	<0,1
Stabiliser (HM-pectin)	0,3	0,3
Acidifier (citric acid)	Nihil	Nihil
lactium™ (0,06%)	0	0,06
Total	100	100

2.1.2. Analysis

- pH
The pH of the beverages was measured at 20 °C with a glass electrode.
- Stability
The stability of the drinks was visually examined after one, two and four weeks of storage at 7°C by measuring serum (whey) layer (relatively transparent top layer) and sediment (white layer on the bottom).
- Microbial stability
The drinks were tested on yeasts, *E. coli* and total bacterial count directly after production.

2.1.3. Sensory properties

The sensory panel for this project consisted of 11-12 assessors of NIZO food research, who were all experienced in the evaluation of liquid food products. The products were judged in a triangle test. The choice was motivated by describing the differences. The products were tested after one week of storage at 7°C. The mango fruit was tested in non-transparent cups, so the product was not visible. The drinks were presented in non-transparent beakers sealed with an aluminium cap with an inserted straw. The products were evaluated in the presence of Mrs. S. Cuisenier (Ingredia, France).

2.2. Recovery of αS1-CN-(91-100) peptide

2.2.1. Materials

All solvents used for the extraction and analytical analysis were of HPLC quality and were purchased from J.T.Baker (Deventer, The Netherlands). Lactic acid was from Analar BDH (90% solution).

2.2.2. Sample preparation method

αS1-CN-(91-100) peptide was extracted from drinks according to the procedure schematically given in Figure 5. First, the samples needed to be acidic (pH _ 4) to be able to fully extract αS1-CN-(f91-100) peptide. Finally, all samples were centrifuged (Eppendorf centrifuge, 5 min, 14,000 rmp (~ 20,000 *g*)) and the supernatant was used directly for analysis.

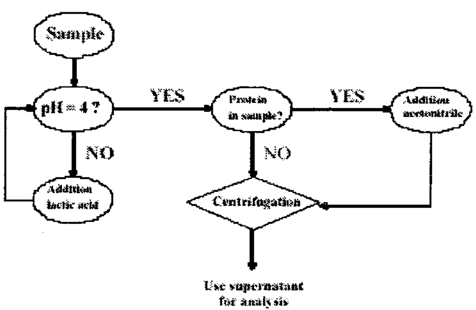

Figure 5 Flow chart sample preparation method to recover α_{s1}-CN (f91-100) peptide for HPLC analysis.

2.2.3. Analysis/Identification of peptide αS1-CN-(f91-100) peptide

The extracted peptides were separated by Reversed Phase HPLC (Polaris C18-A, 5 µm, 150 x 3,0 mm, P/N 2000-150x030, column Varian), maintained at 40°C and coupled to a photodiode array detector (PDA-100, Dionex). The elution was realized using a linear gradient of solvent A (80% water + 20% acetonitrile + 0,1% TFA) and solvent B (35% water + 65% acetonitrile + 0,1% TFA) during 45 minutes at a flow rate of 0,50 mL/min (Table 5). Due to the delay volume (circa 6 mL) of the low pressure gradient pumping system (Waters M600) the αS1- CN-(f91-100) peptide eluted in the isocratic part of the gradient.

Table 5 Gradient used to elute peptide by HPLC. ACN = Acetonitrile.

Time (min)	Flow (ml/min)	Eluent		ACN (%)
		A	B	
0	0,50	82	18	28,1
5	0,50	0	100	65,0
10	0,50	0	100	65,0
15-45	0,50	82	18	28,1

The sample volume injected was 50 μL. The peptide was detected with an UV detector at 275 nm (band width: 5 nm). As a reference 400 nm (band width: 50 nm) was used. To correct for an unknown component in tea an additional wavelength of 350 nm (band width: 5 nm) was used. Using the conditions described the αS1-CN-(f91-100) peptide was eluted at a retention time of 11,7 minutes. The peak was identified as αS1-CN-(f91-100) peptide by liquid chromatography mass spectroscopy (LC-MS) (Addendum 3).

3. RESULTS AND DISCUSSION

3.1. Production and evaluation of drinks

3.1.3. Mango fruit drink (Light)

Mango fruit drink *light* was formulated as follows: 14% (m/m) mango juice concentrate, 0,3% (m/m) pectin, sweetener (nihil), citric acid (nihil) and 86% (m/m) water. The formulation and production of the fruit drink was comparable to commercial products on the market, except the use of pectin in the formulation. This was required to stabilise lactium™.

The visual appearance of the drink with 0,06% lactium™ showed minor differences compared to the reference without lactium™ (Figure 8). The physical stability of both drinks was excellent. After storage during 5 weeks at 7°C both drinks showed no sediment. Furthermore, the mango fruit drink was microbiologically stable as no micro-organisms were detected (total cell count, *E. coli* and yeast negative). Therefore, the mango fruit drink was safe for consumption.

The effect of addition of 0,06% (m/m) on the sensory properties of mango fruit drink is described in Table 8. The addition of lactium™ to the drink did not significantly effect the taste of the fruit drink. Remarks made by the panellists were described differences by taste intensity (general and fruit/mango specific), acid, sweet, mouthfeel. No remarks on increased bitterness or dryer mouthfeel were made of the drink with lactium™.

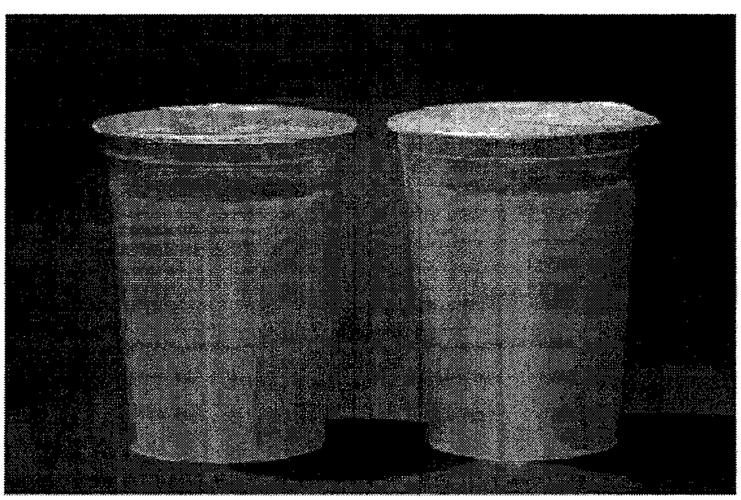

Figure 8 Photographs of produced mango fruit drink without (left) and with Lactium (right).

Table 8 Result of TRIANGLE TEST 4: Fruit drink (mango taste) test. (Alpha risk) * P < 0,05; * *P <
0,01 * * *; P < 0,001

Test	Without Answer	Answers Taken	Answers Right	Significance
FR.-DRINK/FR.-DRINK+LACTIUM™	0	12	6	0,1777

After storage of 5 weeks at 7°C the content of αS1-CN-(91-100) peptide in the mango fruit drink was 92 % (m/m). The acidity and pasteurisation did not affect the content as a full recovery was found (within the error).

It can be concluded that application of αS1-CN-(91-100) peptide in mango fruit drink is not changing taste and stability of the product. The ingredients and processing did not affect the content of the peptide. However, small effects on visual appearance were seen.

3.2. Content of αS1casein-(91-100) peptide

3.2.1. Drinks

For the drinks three different sample preparation methods were developed resulting in a full recovery of αS1casein-(91-100) peptide of >93% in beverages without processing and storage. This method was used to evaluate the content of the peptide after processing and storage of five weeks. The αS1casein-(91-100) peptide was completely (>92%) recovered in tea and fruit drink and partly (70-77%) in UHT-milk and long-life yoghurt drink. The results were discussed previously in this report (section 3.1) and HPLC chromatograms are given in Addendum 6.

Table 10 Recovery of α_{S1}-CN (91-100) peptide in beverage concepts after 5 weeks of storage.

Beverage type	Without processing and storage (%)		After processing and storage 5 weeks at 7°C (%)
	Series 1	Series 2	
Mango fruit drink *light*	97 ± 1	102	92

4. CONCLUSIONS

The aim of this study was to evaluate the taste and content of the anti-stress peptide αS1-CN-(91-100) of the lactium™ hydrolysate in mango fruit drink and to compare the beverage to a reference without lactium™.

Formulation and production of mango fruit drink supplemented with lactium™ was comparable to commercial products on the market. To successfuly stabilise lactium™, pectin was used in the mango fruit drink.

The visual appearance of mango fruit drink was a little bit affected. The peptide αS1-CN-(91-100) of lactium™ did not significantly affect the taste and mouthfeel of the beverage. Remarkably, no bitter off-taste or astringency were noted. The content of the αS1-CN-(91-100) peptide was complete (>92%) in fruit drink