UNIVERSITI PUTRA MALAYSIA

EFFECT OF Lactobacillus casei 431 AND Lactobacillus acidophilus La-5 ON THE PHYSICO-CHEMICAL PROPERTY, SENSORY QUALITY AND VOLATILE COMPOUNDS IN FRESH LIBYAN WHITE CHEESE

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By

FATMA KHALIFA HUSSEIN DABAJ

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Doctor of Philosophy

August 2021

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

EFFECT OF *Lactobacillus casei* 431 AND *Lactobacillus acidophilus* La-5 ON THE PHYSICO-CHEMICAL PROPERTY, SENSORY QUALITY AND VOLATILE COMPOUNDS IN FRESH LIBYAN WHITE CHEESE

By

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August 2021

Chairman : Associate Professor Lasekan Olaniyi, PhD
Faculty : Food Science and Technology

The development of new or improved fermented products is significantly stimulated due to the increasing demand for tasty and healthy foods. Libyan soft white cheese is a very popular traditional dairy product in Libya which is made from fresh or pasteurized milk. There is currently no documented research on the manufacture of fresh Libyan white cheese using Lactic acid bacteria. Thus, the present study is to develop a white cheese using the traditional Libyan manufacturing procedure as well as using *Lactobacillus* spp. Two cultures of lactobacilli (*L. acidophilus* La-5 and *L. casei* 431) were evaluated for their proteolytic activity using spectrophotometric assay, and the volatile organic compounds (VOCs) were specified by using headspace solid-phase microextraction coupled to gas chromatography (HS-SPME-GC) on milk fermentation to select the best strain. Both strains were able to exhibit extracellular proteolytic activities in a broad pH range in a liquid medium. *L. casei* 431 had high extracellular proteolytic activity in an acidic environment (10.50 and 3.31 U/mg in MRS media and whole cow milk, respectively). According to the findings of this study, the analysis of the VOCs produced during the milk fermentation with *L. casei* 431 and *L. acidophilus* La-5 led to the identification of a wide range of VOCs. *L. casei* 431 was able to create more common flavour compounds than *L. acidophilus* La-5, including 2,3-butanedione, 2-heptanone, 3-hydroxy-2-butanone, and 2-nonanone. The white cheese was made by adding *L. casei* 431 in the milk and curd. In this study the physico-chemical, microbial, texture parameters, volatile composition and sensory qualities and the effects of cold storage (5 °C) on the characteristics of white cheese with the different inoculating stage of starter cultures were evaluated. The white cheese samples made with *L. casei* 431 kept the microbial quality during storage study. The *L. casei* 431 resulted in lower springiness, gumminess and chewiness of the white cheese samples compared to the traditional white cheese. Diacetyl, acetoin, 2-heptanone, hexanoic acid and butanoic acid were shown to be major aroma-active components in Libyan white cheese. From flavour profile, the white cheeses were described as cooked, creamy, whey, diacetyl, moisty, sweet, and sour cheese flavour. In addition to that, *L. casei*

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431 could be successfully used to improve the keeping quality of white cheese for 9 days at 5 °C. In this study, the sensory profiles of different pizza cheeses produced with mozzarella cheese and different levels of white cheese were studied using Quantitative Descriptive Analysis (QDA). The appearance, texture and flavour were evaluated. Besides that, the influence of baking methods (microwave and oven) on pizza cheese was also investigated. The principal component analysis showed a good separation of pizza cheese samples. The regression coefficients of scaled and significance indications for sensory characteristics in the different pizza cheese samples showed that the pizza cheese made with 75% mozzarella cheese and 25% white cheese, and baked in the microwave had the best appearance, textural and flavour characteristics. In summary, adding white cheese in pizza cheese gave top sensory characteristics.

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Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

KESAN *Lactobacillus casei* 431 DAN *Lactobacillus acidophilus* La-5 TERHDAP SIFAT FIZIKO-KIMIA, KUALITI DERIA DAN SEBATIAN MERUAP DALAM KEJU PUTIH LIBYAN SEGAR

Oleh

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Pembangunan produk baru atau lebih baik yang ditapai dirangsang dengan ketara disebabkan oleh peningkatan permintaan untuk makanan yang lazat dan sihat. Keju putih lembut Libya ialah produk tenusu tradisional yang sangat popular di Libya yang diperbuat daripada susu segar atau dipasteur. Pada masa ini, tiada penyelidikan yang didokumenkan mengenai pembuatan keju putih Libya segar menggunakan bakteria asid laktik. Oleh itu, kajian ini dibuat adalah untuk memcipta keju putih menggunakan prosedur pembuatan tradisional Libya serta menggunakan *Lactobacillus* spp. Dua kultur lactobacilli (*L. acidophilus La-5* dan *L. casei* 431) telah dinilai untuk aktiviti proteolitik menggunakan ujian spektrofotometri, dan sebatian organik meruap (VOC) ditentukan dengan menggunakan mikroekstraksi fasa pepejal ruang kepala yang digabungkan dengan kromatografi gas (HS-SPME-GC) pada penapaian susu untuk memilih ketegangan terbaik. Kedua-dua strain dapat mempamerkan aktiviti proteolitik ekstraselular dalam julat pH yang luas dalam medium cecair. *L. casei* 431 yang mempunyai aktiviti proteolitik ekstraselular yang tinggi dalam persekitaran berasid (masing-masing 10.50 dan 3.31 U/mg dalam media MRS dan susu lembu keseluruhan). Menurut penemuan kajian ini, analisis VOC yang dihasilkan semasa penapaian susu dengan *L. casei* 431 dan *L. acidophilus* La-5 membawa kepada pengenalpastian pelbagai VOC. *L. casei* 431 mampu mencipta sebatian rasa yang lebih biasa daripada *L. acidophilus* La-5, termasuk 2,3-butanedion, 2-heptanon, 3-hydroxy-2-butanone dan 2-nonanone. Keju putih itu dibuat dengan menambah *L. casei* 431 dalam susu dan dadih. Dalam kajian ini parameter fiziko-kimia, mikrob, tekstur, komposisi meruap dan kualiti sensori dan kesan penyimpanan sejuk (5 °C) ke atas ciri-ciri keju putih dengan peringkat inokulasi berbeza kultur pemula telah dinilai. Sampel keju putih yang dibuat dengan *L. casei* 431 mengekalkan kualiti mikrob semasa kajian penyimpanan. *L. casei* 431 menghasilkan kelembutan, kelekitan dan kekenyalan sampel keju putih yang lebih rendah berbanding keju putih tradisional. Diacetyl, asetoin, 2-heptanon, asid heksanoik dan asid butanoik ditunjukkan sebagai komponen aktif aroma utama dalam keju putih Libya. Daripada profil perisa, keju putih digambarkan sebagai perisa keju yang masak, berkrim, whey, diacetyl, lembap, manis dan masam. Di samping

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itu, *L. casei* 431 boleh digunakan untuk meningkatkan kualiti penyimpanan keju putih selama 9 hari pada suhu 5 °C. Dalam kajian ini, profil sensori keju piza berbeza yang dihasilkan dengan keju mozzarella dan tahap keju putih yang berbeza dikaji menggunakan Analisis Deskriptif Kuantitatif (QDA). Penampilan, tekstur dan rasa dinilai. Selain itu, pengaruh kaedah penaik (gelombang mikro dan ketuhar) terhadap keju piza turut disiasat. Analisis komponen utama menunjukkan pemisahan sampel keju piza yang baik. Pekali regresi bagi petunjuk berskala dan signifikan untuk ciri sensori dalam sampel keju piza yang berbeza menunjukkan bahawa keju piza yang dibuat dengan 75% keju mozzarella dan 25% keju putih, dan dibakar dalam ketuhar gelombang mikro mempunyai ciri penampilan, tekstur dan rasa yang terbaik. Ringkasnya, menambah keju putih dalam keju piza memberikan ciri sensori yang terbaik.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

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LIST OF ABBREVIATIONS

%	Percentage
A_{600nm}	Optical density at wavelength 600 nanometre
AEDA	Aroma extract dilution analysis
ANOVA	Analysis of Variance
AOAC	Association of Official Analytical Chemists
BSA	Bovine serum albumin
°C	Degree Celsius
Ca	Calcium
$CaCl_2$	Calcium chloride
CCRD	Central compound rotational design
CFU	Colony Forming Unit
Cm^2	Square centimetres
CO_2	Carbon dioxide
CRD	Completely Randomized Design
DCM	Dichloromethane
e.g.	Example gratia (for example)
et al.	Et cetera (and company)
FAA	Free Amino Acid
FAO	Food and Agriculture Organization
FDA	Food and Drug Administration
FDM	Fat dry matter
FID	Flame Ionization Detector
g	Gram

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GC	Gas Chromatography
GC-O	Gas Chromatography Olfactometer
h	Hour
HCA	Hierarchical Clustering Analysis
HCL	Hydrochloric acid
HPLC	High Performance Liquid Chromatography
HS	Headspace
ISO	International Organization for Standardization
Kg	Kilogram
L	Litre
L.	*Lactobacillus*
LAB	Lactic Acid Bacteria
M	Molar
Min	Minute
mL	Millilitre
mm	Millimetre
µg	Microgram
µL	Microliter
µm	Micrometre
MNFS	Moisture in Non-Fat Substance
MRS	de Man Rogosa and Sharpe medium
MS	Mass Spectrometry
N	Normality
NaOH	Sodium hydroxide
NaOH	Sodium hydroxide

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ND	Non detected
nm	Nanometre
No.	Number
NSLAB	Nonstarter Lactic Acid Bacteria
OAV	Odour activity value
PCA	Principal Component Analysis
QDA	Quantitative Descriptive Analysis
R^2	Coefficient of determination
RI	Refractive Index
s	Second
SD	Standard Deviation
SNF	Solid-not-fat
SPME	Solid Phase Microextraction
spp.	Species
subsp.	Subspecies
TCA	Trichloroacetic
TPA	Texture Profile Analysis
U	Unit
v/v	volume/volume
VOCs	Volatile Organic Compounds
w/v	weight/volume
WHO	World Health Organization
α	Alpha
β	Beta
κ	kappa

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CHAPTER 1

INTRODUCTION

1.1 Background of the study

In general, global cheese consumption has been increasing significantly, with a ~13 % increase projected between 2021 and 2030 (OECD/FAO, 2021). According to their report, cheese consumption will increase because it has not traditionally been a component of the national diet in some nations. For example, urbanisation and income growth in Southeast Asian countries have led to increase in out-of-home eating, especially fast food like burgers and pizzas. It is worth noting that the epidemic (COVID-19) has increased not just the use of e-groceries and take-out meals in these communities, but also consumer focus on items they believe are healthier or more nutritious. As a result of these growing customer demands, food scientists and cheese producers are focusing their efforts on improving the quality of existing goods or developing new creative products (Lamichhane et al., 2018). Cheese is a biochemically complex substance, and throughout the ripening phase, it undergoes major changes (Hassan et al., 2012). Fresh curds of cheeses varieties have largely similar flavours and aromas. However, during ripening process, the flavouring compounds of each variety are produced (Murtaza et al., 2014). It is now widely agreed that the flavour of certain cheese results from the mixture of the right amounts and quantities of a wide number of multiple substances, also recognised as the principle of component equilibrium (Bertuzzi et al., 2018). The cheese flavour production is a dynamic procedure in which starter cultures, rennet, enzymes of milk and secondary floral are involved in the breakdown of milk proteins, carbohydrates and fats (Bezie, 2019). As a result, the significance of studying cheese flavour is linked to both market acceptance and customer perceptions of cheese's flavour. The fresh cheese flavour, ready to eat cheese, is mostly due to diacetyl and probably acetaldehydes that are the result of the activity of starter bacteria during cheese production. The flavour of mature cheese results from the presence of starter bacteria, milk enzymes, rennet maturation, and secondary floral (Hayaloglu, 2007). Starter cultures play a double function in cheese manufacturing, the production of acid during manufacturing, and growth of flavour at the ripening stage. Until consumption, the majority of rennet cheeses are ripened to obtain attractive organoleptic properties. Ripening comprises a number of diverse biochemical pathways that can be narrowly categorised into the metabolism of lactate, proteolysis and lipolysis. The degree and nature of maturation depend on the temperature and time of storage, the constitution of the cheese (salt and moisture concentration particularly), and the type and behaviour of the enzymes and microorganisms (Fox & McSweeney, 2004).

1.2 Research issues

A number of studies have looked into producing probiotic cheeses out of white cheese and survival of probiotic bacteria in cheese (Buriti et al., 2007; Buriti et al., 2005b; Cárdenas et al., 2014; Dabevska-Kostoska et al., 2015; Dantas et al., 2016; De Souza et

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al., 2008; Haddad et al., 2015 and Kasımoğlu et al., 2004). However, the influence of probiotic bacteria on cheese volatile flavour compounds was not clearly defined. In general, acids, ketones, and alcohols are the major volatiles found to contribute to the flavour of white cheese and they can be detected in different ratios (Medjoudj et al., 2017). However, based on the researchess conducted and the techniques utilized, it appears that the influence of specific chemical classes included in the flavour of cheese were not specified. In addition, starter cultures are not used for the white cheese made in Libya. This cheese is produced without the intentional inclusion of starter culture in the artisanal cheese production; instead, the indigenous milk flora contributes to ripening. In the processing of white cheese, the use of mixed-strain starter colonies comprising of unknown genera of lactic acid bacteria has been used (Effat et al., 2020; Dabevska-Kostoska et al., 2015 and Yerlikaya & Ozer, 2014). In terms of texture and taste, the consistency of cheese made with blended starter cultures differs widely. One of the most critical criteria used in assessing customer preference and approval is cheese flavour. This study was based on the hypothesis that lactobacilli are characterised in their flavour-forming capacity and recognized for their potential to improve some types of cheese flavour when used as adjunct cultures. To date, no research has been conducted on the volatile organic compounds (VOCs) in fresh Libyan white cheese that has LAB. Thus, the present study is to develop a soft white cheese using a traditional Libyan manufacturing procedure with better strains of lactobacilli.

1.3 Aim of the study

Lactobacillus acidophilus and *Lactobacillus casei* are well-known probiotic strain in the dairy industry. However, their capability to contribute to cheese flavour has not been studied. Using probiotics as a starter can improve the flavour, texture, and taste of cheese through proteolytic activity (Abdel-Hamid et al., 2019; Ali et al., 2019 & Raveschot et al., 2018). Based on that, this study was to evaluate the characteristics of Libyan white cheese with probiotics as starter culture, with a view to identifying a suitable starter culture for the white cheeses with enhanced flavour. This research aims to select *lactobacillus* sp. that has high proteolytic activity and high volatilomic potential for manufacturing the fresh Libyan white cheese; and to assess the physicochemical characteristics, sensory quality, and aroma-active compounds when *Lactobacillus* sp. is added into the cow milk before renneting or to the curd before pressing during production and storage; and ultimately, to assess the application of the Libyan white cheese in a food system.

1.4 Research objectives

The principal objectives of the current study were to:

- Evaluate the proteolytic activity and volatilomic potential of the *Lactobacillus acidophilus* La-5 and *Lactobacillus casei* 431 in MRS agar and milk fermentation;

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- Determine the contribution of the selected lactic acid bacterium to the profile of fresh Libyan white cheese volatile organic compounds (VOCs), physico-chemical property and sensory quality during the processing and storage at 5 °C;
- Evaluate the sensorial properties and the application of the produced Libyan white cheese in pizza cheese.

1.5 Significance of the research

This research will help advance the Libyan cheese industry. Thus, it will be incorporated into advanced traditional food products while maintaining and/or improving the sensory characteristics of this type of cheese.

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