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DISSERTATION

TECHNOLOGY OF REDUCED-SALT PORK BATTERS USING SOY PROTEIN ISOLATE AND HIGH PRESSURE PROCESSIN

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АНОТАЦІЯ

Янпінг Лі. Технологія малосолоних свинячих ковбасок із використанням ізоляту соєвого білка та обробкою високим тиском- Кваліфікаційна наукова робота на правах рукопису.

Дисертація на здобуття наукового ступеня доктора філософії за спеціальністю 181 «Харчова технологія», галузь знань 18 «Виробництво та технології». Сумський національний аграрний університет, Суми, 2022.

Дисертацію присвячено технології ковбасок зі свинини зі зниженим вмістом солі, використанням ізоляту соєвого білка та обробкою високим тиском.

У вступі було показано, що надмірне споживання солі може призвести до гіпертонії, серцево-судинних і цереброваскулярних захворювань та інших хронічних захворювань, зменшення споживання солі стало глобальним консенсусом для контролю хронічних захворювань. Всесвітня асамблея охорони здоров’я офіційно прийняла «відносне 30% скорочення споживання солі до 2025 року» як одну з дев’яти добровільних глобальних цілей щодо профілактики та боротьби з неінфекційними захворюваннями. Наразі майже половина країн світу запустили національні програми або дії та розробили власні промислові настанови щодо зменшення споживання солі. Проте традиційні емульсійні м’ясні продукти містять більшу кількість солі, оскільки розчинення розчинного в солі білка при високій концентрації солі (> 0,3 моль/л) є ключовим кроком у формуванні якісної структури гелю. Необхідно знайти нові технології для емульсійних м’ясних продуктів зі зниженим вмістом солі, які могли б вирішити проблему емульсійних м’ясних продуктів, які мають вищі втрати при варінні та гірші властивості гелю при зниженому вмісті солі.

Обробка високим тиском може ефективно змінити структуру білків і техно-функціональні властивості м’ясних продуктів, а також зберегти поживність і смак. Здатність обробки високим тиском інактивувати мікроорганізми та денатурувати білки відома вже понад сто років. Зміни в білкових структурах, конформаціях і властивостях гелю тісно пов’язані з рівнями тиску, часом і температурою, тому визначення оптимальних умов обробки високим тиском є важливим напрямком

досліджень. Застосування обробки високим тиском відкриває деякі цікаві можливості для обробки м'язових харчових продуктів, наприклад, високий тиск може впливати на текстуру та гелеутворювальні властивості м'ясного тіста та міофібрилярних білків, м'якість, колір та інші властивості м'язів. Вплив обробки на продукти на основі м'язів в основному залежить від впливу тиску, часу та температури на відповідні термодинамічні та транспортні властивості м'ясних систем. Зокрема, обробка високим тиском покращує властивості м'язів, подрібненого м'яса та міофібрилярних білків, а використання обробки помірним тиском м'яса, попередньо застиглого, має потенціал, оскільки м'ясо буде ніжним і матиме приємний колір. Обробка високим тиском при оптимальних параметрах процесу покращує водоутримувальну здатність і текстуру подрібненого м'яса, але продукти при цьому не мають готового вигляду та є ймовірність прискореної втрати смаку. Водночас обробка помірним тиском, впливаючи на нековалентні та ковалентні зв'язки і конформацію міофібрилярних білків, водоутримувальну здатність і текстуру міофібрилярних білків, покращує якість м'ясних продуктів зі зниженим вмістом солі.

Вміст білка в ізоляті соєвого білка становить більше 90%, тому це високоякісна рослинна білкова харчова сировина. Його функціональні властивості можна розділити на три категорії: властивості поверхні розділу, властивості гідратації та властивості, пов'язані з білок-білковими взаємодіями, включаючи преципітацію, агрегацію та властивості гелю. Впродовж останніх років на цій основі сотні країн світу розробили тисячі харчових продуктів, що містять соєвий білок. Глобулін соєвого білка має тісно глобулярну структуру, невелику молекулярну масу, активні групові пакети всередині молекули; існуючи методи його модифікації не дозволяють ефективно змінити його структуру та покращити функціональні властивості. Зміни техніко-функціональних властивостей соєвого білка (властивості емульсії, здатність утримувати воду та властивості гелю) під час обробки високим тиском збільшуються або зменшуються в залежності від зміни рівнів тиску, часу та температури. Соєвий білок та його модифіковані продукти завдяки своїм функціональним властивостям широко використовуються при виготовленні м'ясних та молочних продуктів, протеїнових напоїв, хлібобулочних виробів та інших харчових продуктів. Вони відіграють важливу роль у доповненні білка, доповнюючи харчування декількома видами білка, зменшуючи споживання тваринного білка та надаючи харчовим продуктам функції охорони здоров'я. Зокрема, правильна обробка соєвого білка тиском, впливаючи на нековалентний зв'язок, ковалентний зв'язок і конформацію білка, підвищує водоутримувальну здатність, покращує властивості гелю та емульсії, а також знижує алергенність соєвих білків у дитячих сумішах.

Отже, застосування обробки високим тиском і використання ізоляту соєвого білка для модифікації властивостей м'ясних продуктів підвищує водо- і жироутримувальну здатність і якість свинячого фаршу зі зниженим вмістом солі, а також відповідає основному принципу обробки харчових продуктів. Таким чином, було доведено доцільність використання комбінацій обробки високим тиском і використання ізоляту соєвого білка для виробництва свинячого фаршу зі зниженим вмістом солі для покращення здатності утримувати воду та жир та якості продукту в цілому. Дані факти є теоретичним підґрунтям для наших експериментальних досліджень.

Мета дослідження – наукове обґрунтування та розробка виробництва та переробки малосольних виробів із свинячого фаршу за технологією обробки високим тиском і додаванням ізоляту соєвого білка, а також вивчення механізму отримання малосольних виробів із свинячого фаршу під високим тиском при додаванні ізоляту соєвого білка. Предметом дослідження є охолоджена свинина *longissimus lumborum*, свинячий міофібрилярний білок, свинячий фарш зі зниженим вмістом солі, ізолят соєвого білка, параметри обробки високим тиском, комбінація високого тиску та ізоляту соєвого білка, пастеризований свинячий фарш зі зниженим вмістом солі, виготовлений за допомогою високого тиску, використанням ізоляту соєвого білка і подальшим зберіганням в охолодженому стані. Об'єктом дослідження є виробництво свинячого фаршу зі зниженим вмістом солі за технологією обробки високим тиском і поєднанням ізоляту соєвого білка, який має кращу водоутримувальну здатність, гелеподібні властивості, сенсорну якість і триваліший термін зберігання, що є новий методом виробництва високоякісних виробів зі свинячого фаршу зі зниженим вмістом солі, наприклад сосиски та фрикадельки.

Другий розділ містить опис методологічних підходів, використаних в дисертаційній роботі та включає теоретичні дослідження, опис сировини та інгредієнтів, методів експерименту, практичне тестування, просування технології та підпорядковані різні методи дослідження для вирішення наукової проблеми та досягнення мети, поставленої в дисертаційній роботі.

Третій розділ присвячено експериментальним дослідженням в яких, як сировину, використовували охолоджену свинину та ізолят соєвого білка, готували три зразки фаршу з 0%, 2% та 4% ізоляту соєвого білка при 200 МПа протягом 10 хвилин відповідно. Було проаналізовано зміни кольору, виходу при варінні, стабільність емульсії, реологічні властивості і вторинну структуру білка свинячого фаршу (з 1% хлориду натрію), обробленого високим тиском з різним вмістом ізолята соєвого білка, вплив обробки високим тиском і використання соєвого білка. Досліджено комбінації ізолятів на водоутримувальну здатність, техніко-функціональні властивості та білкову конформацію свинячого кляру зі зниженим вмістом солі. Результати показали, що вплив обробки високим тиском і поєднання ізоляту соєвого білка на властивості гелю, реологічні властивості та конформацію білка свинячого фаршу істотно відрізнявся. Значення pH, L* і b*, продуктивність і твердість при варінні, в’язкість і розжовуваність, а також значення G' при 80 °C були значно вищими ($P < 0{,}05$) при додаванні ізоляту соєвого білка, але значення a*, TR, WR і FR були значно знижені ($P < 0{,}05$). Водночас значення TR, WR і FR, G' при 80 °C були значно знижені ($P < 0{,}05$) зі збільшенням ізоляту соєвого білка. Таким чином, додавання 2% ізоляту соєвого білка може покращити водоутримувальну здатність і текстуру свинячого фаршу, обробленого тиском 200 МПа. Результат ЯМР у слабкому полем показав, що представлені зразки, виготовлені з ізоляту соєвого білка, були міцно зв’язані та мали гарну гелеву структуру. Початковий час релаксації T2b, T21 і T22 був меншим ($P < 0{,}05$) у представлених зразках свинячого фаршу з ізолятом соєвого білка, ніж у зразку без ізоляту соєвого білка, і представлені зразки мали найменші пікові співвідношення T22, і найбільше пікове співвідношення T21. Результат спричинив зменшення кількості води, тісно пов’язаної з білками та макромолекулярними компонентами, зниження рухливості води та збільшення водоутримувальної здатності та вмісту зв’язаної води. Доданий ізолят соєвого білка та обробка високим тиском сповільнили термічну денатурацію білків м’яса та зменшили ефект попереднього гелю, спричинений денатурацією хвостів міозину з 53 °C до 59 °C. Структура α-спіралі змінилася на β-пластини, β-виток і структуру випадкової спіралі, коли додали ізолят соєвого білка, і вміст випадкової структури спіралі значно збільшився (P <0,05) зі збільшенням ізоляту соєвого білка. Зокрема, вміст випадкової структури спіралі значно збільшився (P <0,05) з 11,42 ±0,26% до 14,22±0,29% зі збільшенням ізоляту соєвого білка. Загалом, додавання 2% ізоляту соєвого білка може покращити техніко-функціональні властивості та стабільність емульсії свинячого фаршу зі зниженим вмістом солі при обробці високим тиском.

У четвертому розділі представлено результати власного дослідження впливу ізоляту соєвого білка на властивості гелю та водоутримвальну здатність міофібрилярного білку свинини зі зниженим вмістом солі (1% хлориду натрію) під час обробки високим тиском. Свинячий міофібрилярний білок та ізолят соєвого білка використовували як сировину, і три системи міофібрилярного свинячого білка зі зниженим вмістом солі з 0%, 2%, 4% ізоляту соєвого білка готували при 200 МПа протягом 10 хвилин відповідно. Вимірювали текстуру, реологічні властивості, сульфгідрильні групи та стан розподілу води міофібрилярних білкових систем свинини зі зниженим вмістом солі з різним ізолятом соєвого білка (0,2% та 4%) під час обробки високим тиском (200 МПа, 10 хв). Результати показали, що значення L*, вихід при варінні, твердість, загальний і реактивний сульфгідрил, поверхнева гідрофобність і значення G' при 80 °C свинячого міофібрилярного білка були значно збільшені (P <0,05) при додаванні ізоляту соєвого білка; однак пружність, когезія та розжовуваність гелів з 4% ізолятом соєвого білка були нижчими, ніж у гелів з 2% ізолятом соєвого білка. Три системи свинячого міофібрилярного білка зі зниженим вмістом солі показали типову криву G' свинячого міофібрилярного білка після обробки високим тиском з трьома фазами, очевидними від 20 °С до 80 °С. При 80 °С значення G′ зразків, що досліджувалися, становили 2380 Па, 2791 Па та 3113 Па відповідно. Реологічні дані показали, що термічна стабільність міофібрилярного білка була підвищена при додаванні ізоляту соєвого білка. Початковий час релаксації T2b, T21 і T22 був скорочений (P <0,05) при додаванні ізоляту соєвого білка, але він зменшувався зі збільшенням ізоляту соєвого білка; тим часом, пікові співвідношення P2b і P22 були значно знижені (P < 0,05) при додаванні ізоляту соєвого білка, тоді як пікове співвідношення P21 було значно збільшено (P < 0,05), що означає, що вміст зв'язаної і вільної вода знизився, а іммобілізованої - збільшився. Загалом, 2% ізоляту соєвого білка може покращити характеристики гелю та водоутримувальну здатність свинячого міофібрилярного білка при значеннях тиску обробки нижче 200 МПа.

П’ятий розділ містить матеріали, щодо експериментальних досліджень, в яких як сировину використовували м’ясо свинини та соєвий білковий ізолят, готували п’ять свинячих зразків зі зниженим вмістом солі (1% хлориду натрію) з 2% соєвого білкового ізоляту та обробляли тиском 0,1-400 МПа протягом 10 хвилин при 10±2 °С відповідно. Було вивчено вплив різних тисків на техніко-функціональні властивості, розподіл та рухливість води у свинячому фарші зі зниженим вмістом солі, за умови додавання ізоляту соєвого білка, а також pH, властивості гелю, реологію. Результати показали, що порівняно зі зразком 0,1 МПа pH, вихід при варінні, властивості текстури та значення G′ при 80 °С були значно збільшені (P <0,05) у фарші, обробленому різними тисками від 100 МПа до 400 МПа, відповідні початкові періоди релаксації T2b, T21 і T22 були швидшими (P <0,05), що означає, що вода у приготованих зразках, оброблених різним тиском, була краще зв’язана. При цьому всі криві змін на G′ були подібними і мали типову криву динамічної реології з трьома фазами, спричиненими денатурацією білків м’яса під час нагрівання. При цьому зразки, оброблені 200 МПа та 300 МПа, мали найвище значення L*, вихід при варінні, властивості текстури та значення G′ при 80 °С, тоді як початковий період релаксації T2b, T21 та T22 був найкоротшим (P <0,05) і пікове співвідношення P21 було найбільшим, а P22 – найменшим. Навпаки, зразки 0,1 МПа і 100 МПа мали найменше пікове співвідношення P21 і найбільше пікове співвідношення P22. Таким чином, надмірний тиск призвів до погіршення структури гелю та переміщення більшої кількості води, що призвело до збільшення вільної води та зменшення іммобілізованої води. Загалом, обробка тиском 200 МПа та 300 МПа може покращити гелеподібні властивості фаршу зі зниженим вмістом солі з ізолятом соєвого білка, знизити рухливість води та збільшити іммобілізовану воду.

Шостий розділ присвячено експериментальним дослідженням в яких, як сировину, використовували м'ясо свинини та соєвий білковий ізолят і готували два пастеризовані свинячі зразки зі зниженим вмістом солі (1% хлориду натрію) з соєвим білковим ізолятом (0 і 2%), які обробляли при 200 МПа протягом 10 хвилин при 10±2 °C відповідно до розробленої апаратурно-технологічної схеми. Вплив додавання ізоляту соєвого білка у поєднанні з обробкою високим тиском на якість гелю пастеризованого свинячого фаршу зі зниженим вмістом солі, що зберігався при холодній температурі, вивчали шляхом аналізу змін у загальних втратах при зберіганні, TBARS, pH, характеристиках кольору та текстури пастеризованого свинячого фаршу зі зниженим вмістом солі та з різним вмістом ізоляту соєвого білка, обробленого тиском 200 МПа під час холодного зберігання (0-60 днів). Результати показали, що втрати при зберіганні, TBARS, pH, значення L* і твердість зразків значно зросли ($P < 0,05$) зі збільшенням часу холодного зберігання, за винятком зразків з 2% ізолятом соєвого білка, які зберігалися протягом 60 діб. При цьому, протягом перших 30 діб не було виявлено показників будь-якої патогенної мікрофлори, але які були зареєстровані, коли тривалість зберігання збільшилася до 60 діб. Діапазон коливань втрат при зберіганні, мікробіологічні показники, TBARS, pH, кольору та текстури для зразків без ізоляту соєвого білка був більшим, ніж для 2% ізоляту соєвого білка. Загалом додавання 2% ізоляту соєвого білка може покращити властивості гелю, здатність утримувати воду та жир і гальмувати розмноження мікробів у пастеризованому продукті зі зниженим вмістом солі під час холодного зберігання. Зокрема, застосовуючи технологію додавання ізоляту соєвого білка та обробку високим тиском для виробництва пастеризованого свинячого кляру зі зниженим вмістом солі, його вихід при варінні, властивості текстури та структуру гелю були значно збільшено ($P < 0,05$), а також успішно зменшено вміст солі до 1 %.

Відповідно до вищезазначених досліджень розроблена Технологічна схема виробництва пастеризованого малосолоного фаршу із свинини за технологією додавання ізоляту соєвого білка та обробки високим тиском, що є основою для її популяризації та застосування на підприємствах.

За розробленою технологією здійснено масове виробництво на 3 заводах у Китаї для виробництва емульсійних м'ясних продуктів зі зниженим вмістом солі (1% хлориду натрію), таких як фрикадельки, тайванська ковбаса та ковбаса для сніданку. Впровадження технології для виробництва пастеризованого свинячого фаршу зі зниженим вмістом солі дало можливість значно збільшити його вихід, покращити текстуру та структуру гелю, а вміст солі знизити до 1%. Також технологія показала високу соціально-економічну ефективність. Наприклад, виробництво 1 тонни продукту, порівняно з традиційною обробкою, може приблизно на 20-30 кіловат-годин зменшити споживання електроенергії та на 0,8-1,5 тонни - використання проточної води; вміст солі в цьому продукті становить приблизно 1%, що приблизно на 40% нижче, ніж у традиційних продуктах. Згідно з нашими розрахунками, коли споживачі з'їдають 100 г такого продукту, це може зменшити споживання солі на 0,8 г, а вміст білка збільшити на 0,60%~0,85%; для виробництва тонни продукту потрібно на 5%~8% менше часу, що призводить до підвищення продуктивності, збільшення використання цеху та обладнання та зниження амортизації цеху та обладнання; вихід продукту підвищився на 3,66%~5,53%. Нарешті, виготовлення 1 тонни фрикадельок може заощадити 96,42–118,66 дол. США; виробництво 1 тони тайванської ковбаси – $80,37~$96,07; виробництво 1 тони тайванської ковбаси – 96,42–103,12 дол. З вищезазначеного випливає, що м'ясні ковбаски зі свинини з ізолятом соєвого білка, оброблені високим тиском, доцільно використовувати для зниження вмісту натрію в м'ясопереробній промисловості.

У Додатку наведено ключові моменти технології виробництва фаршу зі свинини зі зниженим вмістом солі за технологією високого тиску та поєднання ізоляту соєвого білка, а також Свідоцтва з 3 заводів у Китаї (Henan Zhongpin Food Industry Co. LTD; Hua County Ji Xianda Food Co. LTD; Nanjing Huang Professor Science and Technology Food Co. LTD), що підтверджують виробництво емульсійних м’ясних продуктів зі зниженим вмістом солі (1% хлориду натрію), таких як фрикадельки, тайванська ковбаса та ковбаса для сніданку.

Ключові слова: ізолят соєвого білка, високий тиск, фарш зі свинини, гель, знижений вміст солі, реологічні властивості, реакційноздатний сульфгідрил, вторинна структура, стабільність емульсії, ЯМР у слабкому полі.

ANNOTATION

Yanping Li. Technology of reduced-salt pork batters using soy protein isolate and high pressure processing - Qualifying scientific work on the rights of the manuscript.

Thesis for a Ph.D. Degree specializing in 181 «Food Technology», subject area 18 «Production and technology». Sumy National Agrarian University, Sumy, 2022.

The dissertation is devoted to the technology of reduced-salt pork batters using soy protein isolate and high pressure processing.

In the introduction obtained that excessive salt intake could lead to hypertension, cardiovascular and cerebrovascular diseases and other chronic diseases, the salt reduction has become a global consensus for the control of chronic diseases. The World Health Assembly formally adopted "a relative 30% reduction in salt intake by 2025" as one of nine voluntary global targets for the prevention and control of Non-communicable diseases. At present, nearly half of the countries in the world have launched national salt reduction guidelines or actions, and developed their own industrial salt reduction guidelines. However, the traditional emulsion meat products contain higher salt, because salt soluble protein dissolution at a high salt concentration (> 0.3 mol/L) is a key step in the formation of the good gel structure. Directly decreases the concentration of salt, reduces the amount of salt soluble protein extraction and dissolution, degradation of heat-induced meat gel structure. It is necessary to find new technologies for reduced-salt emulsion meat products that can solve the problem of the emulsion meat products having a higher cooking loss, and worse gel properties when reduced salt content.

The high pressure processing can effectively change the protein structures and techno-functional properties in the meat products, and retain the nutrition and flavour. The ability of high pressure processing to inactivate microorganisms and denature proteins has been known for over one hundred years. The changes in protein structures, conformations, and gel properties are closely related to the pressure levels, time and temperature, so the high pressure processing condition is an important research direction. The application of high pressure processing offers some interesting opportunities in the processing of muscle-based food products, such as the high pressure can affect the texture and gel-forming properties of meat batters and myofibrillar proteins, the tenderize, colour and other

properties of muscle. The processing effects on muscle based products are highly dependent on the primary effects of pressure, time and temperature on the relevant thermodynamic and transport properties of meat systems. Especially, high pressure processing improves the properties of muscle, comminuted meat and myofibrillar proteins, and the use of moderate pressure treatment of pre-rigor meat seems to have potential since the meat will be tender and look normal colour. Reasonable high pressure processing enhances the water holding capacity and texture of comminuted meat, but the products lack the cooked appearance and potential for accelerated loss of flavour. Meanwhile, it improves the quality of reduced-salt meat products through affecting the non-covalent bond, covalent bond and protein conformation of myofibrillar proteins, the water holding capacity and texture of myofibrillar proteins will be increased produce by the moderate pressure treatment.

The protein content of soy protein isolate is more than 90%, it is a high-quality plant protein food raw material. Its functional properties can be divided into three categories: interface properties, hydration properties, and properties related to protein-protein interactions, including precipitation, aggregation, and gel properties. On this basis, hundreds of countries in the world have developed thousands of food products containing soy protein in recent years. The globulin of soy protein has a closely globular structure, the molecular weight is small, and active group packages within the molecule, and some methods of modification are difficult to effectively change the structure and improve its functional characteristics. The changes in techno-functional properties of soy protein by high pressure processing, such as emulsion properties, water holding capacity, and gel properties are increased or decreased with the changes in the pressure levels, time and temperature. Soy protein and its modified products are widely used in meat products, protein drinks, dairy products, baked products and other foods due to their prominent functional properties. They play an important role in supplementing protein, supplementing the nutrition of multiple types of protein, reducing the intake of animal protein, and giving food health care functions. Especially, the proper pressure treatment of soy protein increases the water holding capacity, improves gel and emulsion properties by affecting the non-covalent bond, covalent bond and protein conformation, and also reduces the allergenicity of soy proteins in infant formula.

Thus, the application of high pressure processing and soy protein isolate to modify the properties of meat products, increases the water- and fat- holding capacity and qualities of reduced-salt pork batters, and it also meets the basic principle of food processing. Therein, the relevance and feasibility of using high pressure processing and soy protein isolate combinations for producing reduced-salt pork batters to improve the water- and fat- holding capacity, and qualities have been proved. The result provides a theoretical basis for our experimental design.

Purpose of the research is scientific substantiation and development of the production and processing of reduced-salt pork batter products using the technology of high pressure processing and soy protein isolate combination, and to explore the mechanism of reduced-salt pork batter products by high pressure processing and soy protein isolate combination. Subject of research contain chilled pork *longissimus lumborum*, pork myofibrillar protein, reduced-salt pork batter, soy protein isolate, high pressure processing, high pressure and soy protein isolate combination, pasteurized reduced-salt pork batter produced using high pressure and soy protein isolate combination and stored at cold temperature. Object of research is the production of reduced-salt pork batter using the technology of high pressure processing and soy protein isolate combination, which has better water holding capacity, gel properties, sensory quality and longer shelf-life, obtains a novel method to produce high quality reduced-salt pork batter, such as reduced-salt sausages and meatball.

In the second section, the methodological approaches adopted in the dissertation work include theoretical research, raw materials and ingredients, experiment methods, practical testing, technology promotion, and subordinate various methods of research for the only goal decision set in the dissertation work scientific problem.

In the third section, the chilled pork meat and soy protein isolate were used as raw materials, three batters with 0%, 2%, and 4% soy protein isolate were prepared under 200 MPa for 10 min, respectively. The changes of colour, cooking yield, emulsion stability, rheological property and protein secondary structure attributes of pork meat batters (1%

sodium chloride) treated by high pressure processing with different soy protein isolate were analysed, the effect of high pressure processing and soy protein isolate combinations on the water holding capacity, techno-functional properties and protein conformation of reduced-salt pork meat batters were investigated. The result showed that the effect of high pressure processing and soy protein isolate combinations on the gel properties, rheological property and protein conformation of pork batters were significant differences. The pH, L^* and b^* values, cooking yield and hardness, cohesiveness and chewiness, and G' values at 80 °C were significantly increased (P < 0.05) when soy protein isolate was added, but the a^* value, TR, WR and FR were significantly decreased (P < 0.05). Meanwhile, the TR, WR and FR, G' values at 80 °C were significantly decreased (P < 0.05) with the increase in soy protein isolate. Thus, the 2% soy protein isolate addition could improve the water holding capacity and texture of pork batters treated under 200 MPa. The result of low-field NMR showed that the cooked pork batters made with soy protein isolate were bound tightly, because of the changes of fast relaxing protein and slowly relaxing water protons. The initial relaxation times of T_{2b}, T_{21} and T_{22} were quicker (P < 0.05) in the cooked pork batters with soy protein isolate than that of the sample without soy protein isolate, and the cooked pork batters had the smallest peak ratios of T_{22}, and the largest peak ratio of T_{21}. The result caused the water tightly associated with protein and macromolecular constituents to decrease, the mobility of water was reduced, and increased the water holding capacity and bounded water content. Added soy protein isolate and treated by high pressure processing delayed the thermal denaturation of meat proteins and declined the pre-gel effects generated by the denaturation of myosin tails from 53 °C to 59 °C. The α-helix structure changed into β-sheet, β-turn and random coil structures when soy protein isolate was added, and the random coil structure content was significantly increased (P < 0.05) with the increase in soy protein isolate. Especially, the content of random coil structure was significantly increased (P < 0.05) from 11.42 ±0.26% to 14.22±0.29% with the increase in soy protein isolate. Overall, the 2% soy protein isolate addition could improve the techno-functional properties and emulsion stability of reduced-salt pork meat batters treated with high pressure processing.

The fourth section presents the results of our own research on the effects of soy protein isolate on gel properties and water holding capacity of reduced-salt (1% sodium chloride) pork myofibrillar protein under high pressure processing. The pork myofibrillar protein and soy protein isolate were used as raw materials, and three reduced-salt pork myofibrillar protein systems with 0%, 2%, 4% soy protein isolate were prepared under 200 MPa for 10 min, respectively. The texture, rheological property, sulfhydryl groups, and the water distribution state of reduced-salt pork myofibrillar protein systems with different soy protein isolate (0, 2% and 4%) under high pressure processing (200 MPa, 10 min) were measured. The result showed that the L^* value, cooking yield, hardness, total and reactive sulfhydryl, surface hydrophobicity, and the G' value at 80 °C of pork myofibrillar protein were significantly increased ($P < 0.05$) when soy protein isolate was added; however, the springiness, cohesiveness, and chewiness of gels with 4% soy protein isolate were lower than of gels with 2% soy protein isolate. Three reduced-salt pork myofibrillar protein systems exhibited a typical G' curve of pork myofibrillar protein after high pressure processing with three phases evident from 20 °C to 80 °C. At 80 °C, the G′ values of the treatments were 2380 Pa, 2791 Pa, and 3113 Pa, respectively. The rheological findings indicated that the thermal stability of the myofibrillar protein was increased when soy protein isolate was added. The initial relaxation times of T_{2b}, T_{21}, and T_{22} were shortened ($P < 0.05$) when soy protein isolate was added, but they were decreased with the increase in soy protein isolate; meanwhile, the peak ratios of P_{2b} and P_{22} were significantly decreased ($P < 0.05$) when soy protein isolate was added, whereas the peak ratio of P_{21} was significantly increased ($P < 0.05$), implying that the bound and free water was declined, and the immobilized water was increased. Overall, the 2% soy protein isolate could enhance the gel characteristics and water-holding capacity of pork myofibrillar protein under 200 MPa.

In the fifth section, the pork meat and soy protein isolate were used as raw materials, five reduced-salt (1% sodium chloride) pork batters with 2% soy protein isolate were prepared under 0.1-400 MPa for 10 min at 10±2 °C, respectively. The effects of different pressures on the techno-functional properties, water distribution and mobility of reduced-salt pork batters supplemented with soy protein isolate were examined, and the pH, gel properties, rheology, water distribution and mobility of reduced-salt pork batters were measured. The result showed that compared with the sample of 0.1 MPa, the pH, cooking yield, texture properties and G′ values at 80 °C were significantly increased ($P < 0.05$) in the batters treated by different pressures from 100 MPa to 400 MPa, a corresponding of the initial relaxation times of T_{2b}, T_{21} and T_{22} were faster ($P < 0.05$), implying that the water in the cooked batters treated by different pressures were tied closer. Meanwhile, all the curves of the changes on G′ were similar, and they had a typical curve of dynamic rheology with three phases caused by the meat proteins denaturation during heating. Therein, the samples treated by 200 MPa and 300 MPa had the highest L^* value, cooking yield, texture properties and G′ values at 80 °C, while the initial relaxation times of T_{2b}, T_{21} and T_{22} were shortest ($P < 0.05$), and the peak ratio of P_{21} was the largest and the P_{22} was the smallest. To the contrary, the samples of 0.1 MPa and 100 MPa had the smallest peak ratio of P_{21} and the largest peak ratio of P_{22}. Thus, excessive pressures led to the gel structure being worse and more water moving, causing the free water to increase and the immobilized water to decrease. Overall, treatment by 200 MPa and 300 MPa could improve the gel properties of reduced-salt batters with soy protein isolate, lower the water mobility and increase the immobilized water.

In the sixth section, the pork meat and soy protein isolate were used as raw materials, and two pasteurized reduced-salt (1% sodium chloride) pork batters with soy protein isolate (0 and 2%) were prepared under 200 MPa for 10 min at 10±2 °C according to the operation key points of “Production process and operation key points of produce reduced-salt pork batter using the technology of high pressure and soy protein isolate combination”, respectively. The effects of soy protein isolate and high pressure processing combined on gel qualities of pasteurized reduced-salt pork batter stored at cold temperature were studied, through analysing the changes in storage loss, total, TBARS, pH, colour and texture characteristics of pasteurized reduced-salt pork batters with different soy protein isolate treated under 200 MPa during the cold storage (0-60 days). The results showed that storage loss, TBARS, pH, L^* value, and hardness of pork batters were increased significantly ($P < 0.05$) with the increase in the cold storage time, except the samples with 2% soy protein isolate were stored at the 1st and 30th days. Meanwhile, the total plate count of samples were not detected before the 30th day, while significantly increased at the 60th day. The range of storage loss, microbial, TBARS, pH, colour and texture properties variations from the samples without soy protein isolate were larger than that of 2% soy protein isolate. Overall, adding 2% soy protein isolate could improve the gel properties, water- and fat- holding capacity, and reduce the microbial reproduction of pasteurized reduced-salt pork batter during cold storage. Especially, applying the technology of soy protein isolate and high pressure processing combined to produce the pasteurized reduced-salt pork batter, its cooking yield, texture properties and gel structure were increased significantly ($P < 0.05$), and successfully reduced the salt content to 1%.

According to the above research, “The technological scheme of production in pasteurized reduced-salt pork batter using the technology of soy protein isolate and HPP combined”, “The composition of the technological system and the purpose of its structural operation elements”, and “Technological scheme of pasteurized reduced-salt pork batter using the technology of soy protein isolate and HPP combined” were formulated. They provided a basis for the popularization and application of this technology in enterprises.

The technology has carried out mass production in 3 factories in China to produce reduced-salt (1% sodium chloride) emulsion meat products, such as meatballs, Taiwan sausage and breakfast sausage. Apply the technology to produce the pasteurized reduced-salt pork batter, its cooking yield, texture properties and gel structure were significantly increased, and successfully reduced the salt content to 1%. Meanwhile, the technology has produced good socio-economic effectiveness. Such as, producing 1 ton of product can reduce electricity by about 20~30 kilowatt hours and 0.8~1.5 tons of running water compared with traditional processing; the salt content of this product is approximately 1%, which is about 40% lower than that of traditional products. According to our calculations, when consumers eat 100 g of this type product, which can reduce their salt intake by 0.8 g, and the content of protein is increased by 0.60%~0.85%; it takes 5%~8% less time to produce a ton of product, resulting in higher productivity, increased workshop and equipment utilization, and reduced workshop and equipment depreciation; the cooking yield of product was increased by 3.66%~5.53%. Finally, producing 1 ton of meatballs can save you $96.42~$118.66; producing 1 ton of Taiwan sausage can save you

$80.37~$96.07; producing 1 ton of Taiwan sausage can save you $96.42~$103.12. From the above, we can know that the pork meat batters with soy protein isolate treated by high pressure processing should be adopted for lowering sodium content in the meat processing industry.

The Appendix provides the Operation key points of “Production process and operation key points of produce reduced-salt pork batter using the technology of high pressure and soy protein isolate combination”, and the Application Testify of 3 factories in China (Henan Zhongpin Food Industry Co. LTD; Hua County Ji Xianda Food Co. LTD; Nanjing Huang Professor Science and Technology Food Co. LTD) to produce reduced-salt (1% sodium chloride) emulsion meat products, such as meatballs, Taiwan sausage and breakfast sausage.

Keywords: soy protein isolate, high pressure, pork batter, gel, reduced-salt, rheology properties, reactive sulfhydryl, secondary structure, emulsion stability, low-field NMR.

LIST OF APPLICATION

1. List of published articles

1.1 Published scientific works of main achievements:

1.1.1 In foreign scientific professional journals indexed by Scopus and Web of Science Core Collection

1. **Li, Y**., Sukmanov, V., Kang, Z., & Ma, H. (2020). Effect of soy protein isolate on the techno-functional properties and protein conformation of low-sodium pork meat batters treated by high pressure. Journal of Food Process Engineering, 43(2**). (Web of Science Core Collection, Q3).**

Personal contribution: Conceptualization, Methodology, Validation, Data Curation, Investigation, Writing - original draft, Writing-Review & Editing, Visualization.

2. **Li, Y.,** Kang, Z., Sukmanov, V., & Ma, H. (2021). Effects of soy protein isolate on gel properties and water holding capacity of reduced-salt pork myofibrillar protein under high pressure processing. Meat science, 176, 108471. **(Web of Science Core Collection, Q1).**

Personal contribution: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Writing - original draft

3. **Li, Y.,** Kang, Z., Sukmanov, V., & Ma, H. (2021).Technological and functional properties of reduced-salt pork batter incorporated with soy protein isolate after pressure treatment Running Head: Reduced-salt batters affected by pressures. International Journal of Food Science & Technology.1365-2621. **(Web of Science Core Collection, Q2).**

Personal contribution: Conceptualization, Methodology, Formal analysis, Data Curation, Investigation, Writing - original draft, Writing-Review & Editing.

4.Jing-jie Xie, **Yan-ping Li,** Xiao-Qing Qu, Zhuang-Li Kang. Effects of combined high pressure and temperature on solubility, foaming, and rheological properties of soy 11S globulin. Journal of Food Process Engineering, 2022.**(Web of Science Core Collection, Q3)**

Supervision, Validation, Writing-Review & Editing.

1.1.2 In Ukrainian scientific professional journals indexed by Scopus and Web of Science Core Collection (category A)

1. Valerii Sukmanov, Ma Hanjun, **Yan-ping Li.** (2019). Effect of high pressure and soy protein isolate combinations on the water holding capacity and texture of pork meat batters. Ukrainian Food Journal, 8(2): 284-291. **(Web of Science Core Collection).**

Personal contribution: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Writing - original draft

2. Valerii Sukmanov, Ma Hanjun, **Yan-ping Li.** (2019). Effect of high pressure processing on meat and meat products. A review. Ukrainian Food Journal, 8(3): 448-455. **(Web of Science Core Collection).**

Personal contribution: Conceptualization, Methodology, Formal analysis, Data Curation, Investigation, Writing - original draft, Writing-Review & Editing.

3. **Li, Y.,** Sukmanov, V., Ma, H. (2021).The effect of high pressure on soy protein functional features: A review. Journal of Chemistry and Technologies. 29(1), 77-91. **(Scopus).**

Personal contribution: Conceptualization, Methodology, Formal analysis, Data Curation, Investigation, Writing - original draft, Writing-Review & Editing.

1.1.3 In Ukrainian scientific professional journals (category B)

1. **Yan-ping Li**, Valerii. Sukmanov, Ma Hanjun, Zhuang-li Kang. High Pressure Applications in Low Salt Gel Meat Technologies. A review. Ukrainian Journal of Food Science. 2021, Vol.9, Is.2. pp. 321-327.

Personal contribution: Conceptualization, Methodology, Formal analysis, Data Curation, Investigation, Writing - original draft, Writing-Review & Editing.

1.2 Scientific works certifying that paper materials are recognized:

1. **Li Yanping,** Valerii Sukmanov, MA Hanjun. Effect of high pressure and soy protein isolate combinations on the gel properties and protein conformation of low-sodium pork meat batters. 2019 ASIA - PACIFIC CONGRESS OF MEAT SCIENCE AND TECHNOLOGY, 62.

2. **Li Yanping**, Valerii Sukmanov, MA Hanjun, Kang Zhuang-Li. Effect of sodium alginate in low-fat frankfurters: A physical-chemical and Raman spectroscopy study. 2019 ASIA - PACIFIC CONGRESS OF MEAT SCIENCE AND TECHNOLOGY, 105.

3. **Li Yanping,** Valerii Sukmanov, MA Hanjun. Effect of NaCl and soy protein isolate combinations in frankfurters: A physicalchemical and Low field NMR study. 2019 ASIA - PACIFIC CONGRESS OF MEAT SCIENCE AND TECHNOLOGY, 109.

4. **Li Yanping**, Sukmanov V., Ma Hanjun. The rheological properties of pork meat batters, produced using soy protein isolate and processed by high pressure. Розвиток харчових виробництв, ресторанного та готельного господарств і торгівлі: проблеми, перспективи, ефективність : Міжнародна науково-практична конференція, 15 травня 2019 р. : [тези у 2-х ч.] / редкол. : О. І. Черевко [та ін.]. – Харків : ХДУХТ, 2019. – Ч. 1. – 316, [XII], С 42-44.

5. **Li Yanping**, Ma Hanjun. Effect of high pressure on the water holding capacity of myofibrillar protein gel, Матеріали XV Міжнародного форуму молоді "Молодь і сільськогосподарська техніка у xxi сторіччі", 4-5 квітня 2019р., Харків, С.15.

6. Valerii Sukmanov, Ma Hanjun, **Yan-ping Li.** Effect of high pressure and soy protein isolate combinations on the water holding capacity and texture of pork meat batters. Техника и технология пищевых производств: материалы XIII Междунар. науч.-техн. конф., 23–24 апреля 2020 г., в 2-х т., Могилев / Учреждение образования «Могилевский государственный университет продовольствия» – Могилев: МГУП, 2020. – Т.2– С. 68-70.

7. **Li Yanping**, Sukmanov V.A., Ma Hanjun. Technology of reduced-salt emulsion meat products with the addition of soy protein isolate and high pressure treatment. Матеріали XI Международной научной конференции студентов и аспирантов «техника и технология пищевых производств». «Могилевский государственный университет продовольствия».18-19 апреля 2019 года.

8. **Li Yanping**, MENG Lin, Valerii Sukmanov, MA Hanjun. (2019). Impact of Transglutaminase on Gel Properties of Duck Breast Meat Batter. ASIA-PACIFIC CONGRESS OF MEAT SCIENCE AND TECHNOLOGY, 29.

9. **Li Yanping,** Valerii Sukmanov, KANG Zhuangli, MA Hanjun. (2019). Effects of Pre-emulsified Corn Germ Oil By Soy Protein Isolate on Quality of Chicken Meatball. ASIA-PACIFIC CONGRESS OF MEAT SCIENCE AND TECHNOLOGY, 89.

10. Сукманов В.О., **Ян-Пінг Лі**, Дзі Зенг. Використання високого тиску в технологіях м'яса та м'ясопродуктів. Тренди Lean-виробництва та пакування харчової продукції: матеріали 10-ї Міжнародної спеціалізованої науково-практичної конференції, 15 вересня 2021 р., м. Київ. – Київ, НУХТ, 2021. – С. 11-13.

11. Sukmanov V., Ma Hanjun, **Yan-Ping Li.** Study of the influence of high pressure and the introduction of soy protein isolate on some properties of pork meat batters. P.242-243. Інноваційні аспекти розвитку обладнання харчової і готельної індустрії в умовах сучасності : третя міжнародна науково-практична конференція, 4–6 вересня 2019 р. : [тези доп.] / під заг. ред. Г. В. Дейниченка. – Харків : ХДУХТ, 2019. – 272 с.

12. **Yan-ping Li**, Valerii Sukmanov, Ma Hanjun. The effects of high pressure processing on the meat and meat products. Матеріали XX Міжнародної науково-практичної конференції. "Сучасні напрямки технології процесів переробних і харчових виробництв", 8 листопада, Харків, 2019, С. 94.

13. **Yan-ping Li**, Xinxiang, Valerii Sukmanov, Ma Hanjun. Technological and functional properties of reduced-salt pork batter incorporated with soy protein isolate after high pressure treatment. Інноваційні технології та перспективи розвитку м'ясопереробної галузі («Реалії та перспективи м'ясопереробки»): Програма та тези матеріалів Міжнародної науково-практичної конференції, 15 вересня 2021 р., м. Київ. – К.: НУХТ, 2021 р. – С. 16.

14. Sukmanov V. **Yan-ping Li**, Fang Wang. Operating principles of high pressure technological equipment in food production. Збірник тез доповідей Всеукраїнської науково-практичної конференції «Інноваційні та ресурсозберігаючі технології харчових виробництв» 21 грудня 2021 року, м. Полтава. 163 с.

CATALOGUE

THE LIST OF SYMBOLS

Abbreviation	Full name
AOAC	Association of Official Analytical Chemists
PSE	pale, soft, and exudative
NaCl	sodium chloride
ANS	1-anilinonaphthalene-8-sulphonic acid
TBARS	thiobarbituric acid reactive substances
DNA	deoxyribonucleic acid
7S	β-conglycinin
11S	globulin
SE	standard error
SDS-PAGE	sodium dodecyl sulfate polyacrylamide gel electrophoresis
TPA	texture profile analysis
NMR	nuclear magnetic resonance imaging
SPSS	Statistic Package for Social Science
ANOVA	analysis of variance
WHO	World health organization
TR	total fluid release
WR	water released
FR	fat released
G'	storage modulus
LSD	Least Significant Difference
L^*	light
a^*	red
b^*	yellow
GLM	general linear model
A_w	water activity

INTRODUCTION

Relevance of the topic.

Based on the fact, that excessive salt intake can lead to hypertension, cardiovascular and cerebrovascular diseases and other chronic diseases, the salt reduction has become a global consensus for the control of chronic diseases. The World Health Assembly formally adopted "a relative 30% reduction in salt intake by 2025" as one of nine voluntary global targets for the prevention and control of Non-communicable diseases. At present, nearly half of the countries in the world have launched national salt reduction guidelines or actions, and developed their industrial salt reduction guidelines.

The World Health Organization (WHO) suggested the intake of salt for adults at 5-6 g/ day, and the intake of salt from meat and meat products accounts for 16-25% of the total intake (Zhang et al., 2017). For years, the demand for the emulsion meat products, such as sausages and meatballs, are increasing rapidly, which has enjoyed wide consumer acceptance in certain sections of the global population. However, the traditional emulsion meat products contain higher salt (approximately 2%), because salt soluble protein dissolution at a high salt concentration (> 0.3 mol/L) is a key step in the formation of good gel structure (Kang, Li, Ma, & Chen, 2016).

According to the classical theory of meat gel, salt soluble protein must be extracted and fully dissolved at an enough high concentration of salt to form a good meat gel structure. Directly decreased the concentration of salt, reduce the amount of salt soluble protein extraction and dissolution, degradation of the heat-induced meat gel structure. Therefore, how to reduce sodium chloride and ensure the quality of emulsion meat products is a difficult question. Some methods of reduce-salt have been studied, such as used high pressure processing, and added soy protein isolate (Wang, Zhou, Wang, Li, Xu & Chen, 2020; Li, Sukmanov, Kang & Ma, Kang, Zou, Meng & Li, 2021).

To reduced-salt in emulsion meat products, high pressure processing (high hydrostatic pressure) has been reported by some researchers and achieved better results (Dixon, Rabanser, Dzieciol, Zwirzitz & Wetzels, 2019; Maksimenko, Kikuchi, Tsutsuura & Nishiumi, 2020; Wang, Zhou, Wang, Li, Xu & Chen, 2020; Li, Sukmanov, Kang & Ma, 2020; Li, Kang, Sukmanov & Ma, 2021). However, using high pressure technology alone to reduce salt also has many disadvantages, such as harsh high pressure conditions, and difficulty to improve the texture properties, water- and fat- holding capacity of emulsion products. Thus, the problems still need further study.

The high pressure processing is a kind of physical sterilization technology, it can effectively change the protein structure and techno-functional properties in meat products, and retain the nutrition and flavour. The ability of high pressure processing to inactivate microorganisms and denature proteins has been known for over one hundred years. The changes in protein structures, conformations, and gel properties are closely related to the pressure levels, time and temperature, so the high pressure processing condition is an important research direction.

Due to the structure of protein material component differences appeared different degrees of compression deformation, when the deformation degree was large enough, it may affect the combination between protein molecules formed, and cause the destruction and restructuring, which affected the functional characteristics of the protein. Moreover, protein conformation may bring better functional characteristics due to the sudden release of pressure after pressure was withdrawn (Gao, Wang, Mu, Shi & Yuan, 2018; Xu, Zhang, Wang & Liu, 2019; Wei et al., 2019). The high pressure processing played a key role in forming the quality of the meat products, there were a few reports on reducing sodium chloride in the meat products, but the mechanism of lowering sodium chloride was still not completely understand (Jayathilakan, Sultana & Pandey, 2019).

Now, soybean cultivation has spread all over the world. Because of its high yield, high protein content, rich nutrition and good functional characteristics, which has become an important food resource for human beings, and is one of the world's most important economic crops (Kinsella, 1979; Baum et al., 1998; Erdman, 2000; Shen, Liu, Geng, Zhang, & Liu, 2018; Zhang, Luo, Wang, Li & Liu, 2020). Soy protein, especial soy protein isolate, has good gel properties, water- and fat- holding capacity, and wildly used in the meat products to improve the yield and quality (Brandenburg, Weller & Testin, 2010; Wu, Sun, Bi, Ji & Xing, 2018; Wolf, 2019). Since the addition of soy protein isolate affects the quality of emulsion meat products, the influence of the addition of soy protein isolate on reduced-salt pork batter needs further study, in order to maximize the effect of soy protein isolate.

The changes in techno-functional properties of soy protein isolate by high pressure processing, such as emulsion properties, water holding capacity, and gel properties, were increased or decreased with the changes in pressure levels, time and temperature, following, the rheological properties, gel properties and the content of immobile water were changed. Therefore, the aim of this study is to investigate the application of high pressure processing and soy protein isolate combination to modify the properties of meat products, increase the water holding capacity and texture of cooked gel meat products, and then the use of high pressure and soy protein isolate combinations to emulsion meat products could improve the quality and lower the salt content in the meat industry.

Connection of work with scientific programs, plans, and topics.

The work was carried out in accordance with the main directions of scientific research of the Poltava State Agrarian University on the state budget topic "Innovative and resource-saving technologies of food production" (No. SR 0115U006745), Sumy National Agrarian University within the framework of scientific topics: China Postdoctoral Science Foundation (2016M602237) “Effects of high pressure on gel properties of mixed pork myofibrillar protein and soy protein isolate”, and Natural Science Foundation of Henan Province (212300410344) “Modification of chicken myofibrillar protein with sodium bicarbonate and the mechanism of gel formation”.

The Aim and Objectives of the study.

The purpose of the dissertation work is scientific substantiation and development of the production and processing of reduced-salt pork batter products using the technology of high pressure and soy protein isolate combination, and to explore the mechanism of reduced-salt pork batter products by high pressure and soy protein isolate combination.

To achieve the main goal, it was necessary to solve a number of interrelated tasks:

– to analyze literary sources in accordance with relevant production of reduced-salt pork batter products using the technology of high pressure and soy protein isolate combination, and discuss the feasibility of this technology to produce the reduced-salt pork batter products.

– to investigate the changes in water holding capacity and texture of pork batters (1% sodium chloride) with soy protein isolate (0%, 2% and 4%) used in the high pressure processing (200 MPa, 10 min), obtain the effect of the optimum soy protein isolate addition on water holding capacity and texture of reduced-salt pork batter.

– to study the changes of color, emulsion stability, rheological property and protein secondary structure attributes of pork meat batters (1% sodium chloride) treated by high pressure (200 MPa, 10 min) with different soy protein isolate (0%, 2% and 4%) according to the changes of water holding capacity and texture of reduced-salt pork batter, obtain the effect of the optimum soy protein isolate addition to improving the quality of reduced-salt pork batter.

– to investigate the effects of different pressures (0.1-400 MPa), the pH, gel properties, rheology, water distribution and mobility of reduced-salt pork batters (1% sodium chloride) with soy protein isolate (2%) were studied and analyzed the changes of techno-functional properties, water distribution and mobility of reduced-salt pork batters with soy protein isolate as affected by pressures, and find the optimum pressure level.

– extract myofibrillar protein from the *longus dorsi* muscle of pork, then prepare a mixture of myofibrillar protein (60 mg/ml) and soy protein isolate (0%, 2% and 4%) in a mixed protein solution with 1% sodium chloride under high pressure (200 MPa, 10 minutes). To study the effect of adding various soy protein isolates on the texture, rheology, sulfhydryl groups, and water distribution state of the mixed protein solution to analyze the mechanism of salt reduction using high pressure technology and soy protein isolate combination.

– develop technology to combine high pressure and soy protein isolate for use in the production of reduced salt pork batter (1% sodium chloride), the study changes in storage loss, total mass, TBARS, pH, color and textural characteristics of pasteurized reduced salt pork batter with different soy protein isolate (0 and 2%) processed at 200 MPa during cold storage (60 days), to analyze the effect of soy protein isolate and high pressure on the gel-like properties of pasteurized pork batter with reduced salt content, stored at cold temperature, get quality changes during storage.

– to substantiate the parameters and shelf life of the pasteurized reduced-salt pork batter, and to investigate the stability of basic quality indicators during cold storage.

– to develop and approve normative documents for the production of reduced-salt pork batter using the technology of high pressure and soy protein isolate combination, and make recommendations on its use in factory mass production.

– to determine the socio-economic effectiveness of scientific and technical developments and implement the results of work in practical production.

Object research

– production of reduced-salt pork batter using the technology of high pressure and soy protein isolate combination.

Subject of research

– chilled pork *longissimus lumborum*, pork myofibrillar protein, reduced-salt pork batter, soy protein isolate, high pressure processing, high pressure and soy protein isolate combination, pasteurized reduced-salt pork batter produced using high pressure and soy protein isolate combination stored at cold temperature.

Research methods

Standard physicochemical, texture properties, low field nuclear magnetic resonance, Raman spectroscopy, inoxidizability, sulfhydryl groups, surface hydrophobicity, emulsion stability, rheological property, microbiological, organoleptic, experiment planning methods and mathematical processing of experimental data computer programs.

Scientific novelty of the obtained results

Consists in the theoretical and experimental substantiation of new technologies for the production of reduced-salt pork batter using high pressure and soy protein isolate combination, and obtain the products with lower salt content and higher organoleptic properties and water holding capacity. The main contents are as follows:

– added 2% soy protein isolate and high pressure (200 MPa, 10 min) combinations enabled to production the reduced-salt pork batter with better water holding capacity and texture properties.

– the 2% soy protein isolate addition could improve the pH, L^* and b^* values and emulsion stability of pork meat batters treated by high pressure.

– added soy protein isolate and treated by high pressure delayed the thermal denaturation of meat protein and declined the pre-gel effects generated by the denaturation of myosin tails from 53 °C to 59 °C, and induced the α-helix structure to change into β-sheet, β-turn and random coil structures.

– treated by 200 and 300 MPa could improve the pH, cooking yield, texture properties and G′ values at 80 °C of reduced-salt batters with soy protein isolate (2%), lower the water mobility and increase the immobilized water.

– the 2% soy protein isolate addition could enhance the L^* value, cooking yield, hardness, total and reactive sulfhydryl, surface hydrophobicity, and the G' value at 80 °C of pork myofibrillar protein under 200 MPa. On the other, the thermal stability of the myofibrillar protein increased when soy protein isolate was added, and the water had lower mobility.

– adding 2% soy protein isolate could improve the gel properties, water- and fat-holding capacity, and reduce the microbial reproduction of pasteurized reduced-salt pork batter during cold storage.

– the pork batters with soy protein isolate treated by high pressure processing should be adopted for lowering sodium content in the meat processing industry.

Practical significance of the obtained results

Based on the results of theoretical and experimental studies developed by the technologies of reduced-salt pork batter using the technology of high pressure and soy protein isolate combination.

According to the development and approval of relevant procedures, the “production process and operation key points of produce reduced-salt pork batter using the technology of high pressure and soy protein isolate combination” was formulated.

Base on the situation of the factory, established the shelf life and storage method of reduced-salt pork batter using the technology of high pressure and soy protein isolate combination.

According to market demand and factory requirements, the technology of high pressure and soy protein isolate combination have carried out mass production in 3 factories to produce reduced-salt (1% sodium chloride) emulsion meat products.

Applicant's personal contribution is in planning an experiment, organization and conduct of analytical and experimental research in laboratory and production conditions, analysis, processing and generalization results, formulating conclusions and recommendations, preparing materials for publication, developing and approval of normative documentation, and introduction of new technologies into production.

Publication. Based on the results of the dissertation work, 10 articles were published in scientific journals. 3 articles - in scientific publications are included in the List of scientific professional publications Web of Science Core Collection Q1 - Q3 which taking into account the Order of awarding the degree of Doctor of Philosophy is 6 articles; 3 articles in scientific publications included in the List of scientific professional publications Scopus and Web of Science Core Collection; 1 scientific article in Ukrainian scientific professional journal (category B).

Approbation of dissertation results. The main results of the dissertation were presented at ASIA - PACIFIC CONGRESS OF MEAT SCIENCE AND TECHNOLOGY, (2019); International scientific-practical conference "Development of food production, restaurant and hotel facilities and trade: problems, prospects, efficiency", 2019; XV International Youth Forum "Youth and Agricultural Machinery in the XXI Century" 2019; XI International Scientific Conference of Students and Postgraduates "Technique and Technology of Food Production" of Mogilev State University of Food", 2019; the third international scientific-practical conference "Innovative aspects of the development of equipment for the food and hotel industry in modern conditions", 2019; XX International Scientific and Practical Conference. "Modern directions of technology of processes of processing and food production", 2019; XIII International Scientific and Technical Conference "Technique and Technology of Food Production", Belarus, Mogilev, 2020; 10^{th} International Specialized Scientific and Practical Conference "Trends in Lean Production and Packaging of Food Products: Materials", 2021; International scientific-practical conference "Innovative technologies and prospects for the development of the meat processing industry ("Realities and prospects of meat processing "), 2021; All-Ukrainian scientific-practical conference "Innovative and resource-saving technologies of food production", 2021.

Structure and scope of the dissertation. The dissertation consists of an annotation, introduction, 6 sections, conclusions, and a list of sources used, including 213 references and appendices. Main content dissertation is laid out on 112 pages of printed text, and contains 15 tables, 16 figures.

SECTION 1.

EFFECTS OF HIGH PRESSURE PROCESSING ON THE MEAT, MEAT PRODUCTS AND SOY PROTEIN ISOLATE

High pressure processing (HPP) can be referred to as HPP or hydrostatic technology, the water or other incompressible fluid mediums often act as mediators of pressure. During the HPP, the pressure levels are generally not less than 100 MPa, the commonly used range is 100-1000 MPa and can work in the temperature range of -20 °C to 90 °C. HPP has the advantages of pressure uniform transmission, instantaneous, efficient, low energy consumption, pollution little dyeing, and no obvious effects of low molecular compounds such as Vitamins, pigments and flavor substances, etc. Therefore, HPP technology can develop the appearance and new types of meat with different textures will be available in meat processing and storage. At present, as far as we know, the application of HPP technology in meat processing mainly includes improving meat quality, sterilization and freezing and thawing of meat, such as improving meat tenderness, water and fat holding capacity, fat oxidation, and gel properties. However, in order to realize the large-scale application of HPP technology in meat processing, there are still many problems worthy of in-depth discussion, and the research on these problems may be the key consideration in the future. First, the HPP equipment needs a high investment, which has to solve the problem of high cost, which seriously restricts the promotion of industrialization. Second, the affecting factors of HPP is complex and diverse, including pressure levels, time, temperature, pressure and the characteristics of raw materials, and so on. The effects of HPP current research are not much, and need a lot of research in the long term.

The application of HPP offers some interesting opportunities in the processing of muscle-based food products, such as, the HPP can affect the texture and gel-forming properties of meat batter and myofibrillar proteins, the tenderize, colour and other properties of muscle. The processing effects on muscle based products are highly dependent on the primary effects of pressure, time and temperature on the relevant thermodynamic and transport properties of meat systems. However, the pressure-labile nature of some meat protein systems, such as myosin or myoglobin often limits the range of attractive commercial applications to prefermented and cooked meat products.

1.1 Effects of HPP on meat and meat products

1.1.1 The principle of HPP

The fundamental principles of the hyperbaric technique are the Pascaline law and the Le Chatelier principle. Pascaline law takes advantage of the compression effect of HPP on liquids, which means that the pressure applied to the liquid can be transmitted to all parts of the system instantaneously at the same size. Therefore, dry food, powdery food or granular food should not be used HPP. According to Pascaline law, the effect of HPP is independent of the size, shape and volume of the food. In the process of HPP, the whole food will be treated uniformly, the pressure transfer speed is fast, and there is no pressure gradient. Therefore, the HPP of food is simpler, and the energy consumption is also significantly reduced. According to Le Chatelier principle, the external pressure reduces the volume of the pressurized system and vice versa. Therefore, the physical and chemical reactions in food ingredients will be carried out in the direction of the maximum compression state under the pressure treatment of food. The increase or decrease of the reaction rate constant k depends on whether the “active volume” of the reaction is positive or negative. This means that HPP processed food will force the reaction system to reduce the volume, affecting not only the reaction balance in the food, but also the reaction rate, including chemical reactions and possible changes in molecular conformation.

1.1.2 Effects of HPP on the properties of muscle

Effects of HPP on the pH of muscle. The effects of HPP on the pH of meat depended on pressure levels, time and temperature, muscle type and so on. The fresh meat had a rapid pH decrease and an intense contraction after HPP. The main reason is that pressurization induced contraction causes calcium release stimulating glycolysis, and the changes in activity of phosphorylase, phosphorylase kinase and phosphorylase phosphatase, which breakdown the regulation of glycogen during the HPP. The pH of red meat, such as ovine and bovine muscles were decreased 0.6-0.8 unit after 100-150 MPa, l-5 min at 35 °C. However, the pH of white meat, such as *longissimus dorsi* from rabbit had a larger decrease than the masseter after 10 min pressurisation (Cheftel & Culioli, 1997).

The HPP also affected the ultimate pH of meat. The ultimate pH of pre-rigor pork longissimus increased by 0.48 after HPP at 215 MPa (Souza et al., 2011). Simonin et al. (2012) showed that the HPP of post-rigor muscles increased the ultimate pH of the meat. The differences were caused by the different meat types and pressure conditions.

The post-rigor meat had a slight pH increase after HPP, and the pH increased with the pressure levels increased. The reason might be that the exposure of acidic groups were decreased due to conformational proteins denaturation during the HPP (Poulter et al. 2010). The pH of porcine and bovine M. semimembranosus muscles slightly increased from 5.6 to 5.8 after 400 MPa at 20 °C for 10 min, and the pH of the post-rigor muscle slightly increased from 5.4 to 5.6 under 100 and 400 MPa at 15 °C for 5 min, respectively (Kwiatkowska et al., 2002; Kim et al., 2007). Ma et al. (2019) reported that the pH of yak meat increased with pressure levels increased from 0.1 to 450 MPa. Morton et al. (2017) found that the mean pH of the prime and bull caused a significant increase, and the cow meat had a significant decrease under 175 MPa, and the mean pH of meat from all the animal classes was significantly increased by 250 MPa treatment.

Effects of HPP on the colour of muscle. Meat colour is one of the most important quality properties for the consumers in a purchase situation, which is determined to the consumers purchase it or not. For example, consumers usually like a bright red colour of beef meat, and a stable reddish/pink colour of cured pork products, these were perceived as a sign of freshness (Schulte et al., 1995; Sikes & Tume, 2014). Myoglobin is the most important meat pigment, making up 90-95% of the total pigment content. Its concentration and chemical-physical state have a key steps in the colour of fresh and processed meat (Carlez, Veciana-Nogues, & Cheftel, 1995). The colour changes of meat induced by HPP are basically dependent on three main mechanisms: denaturation of myoglobin, modification or disruption of the porphyrin ring, and changes in the myoglobin redox chemistry (Bak et al., 2017). These were connected with the meat type, pressure conditions, pH, etc. HPP conditions and myoglobin redox form prior to HPP are the main reasons for the colour changes of pressurized meat. At low temperatures, below 300 MPa treatment have minor effects on colour than the other pressures. But the myoglobin is not stable, the denaturation had been found to take place at low pressures (Bak et al., 2012;

2019). Korzeniowski et al. (1999) found that the 28% of myoglobin was denatured after 100 MPa treatment, increasing to 66% denaturation of myoglobin after 400 MPa treatment. Souza et al. (2011) found that the HPP treated *longissimus dorsi, triceps brachii,* and *psoas major* muscles had L^* values that were 3.87, 6.37, and 2.71 units higher than controls; the a^* values for treated *longissimus dorsi* muscles were 0.94 units lower than controls, while treated *triceps brachii* muscles were 0.67 units higher than controls. Due to the denaturation of myoglobin, HPP possibly gives the fresh meat a cooked appearance that does not visually appeal to consumers. Carlez et al. (1995) found that the colour of minced beef was changed into "whitening" when the pressures were over 200 MPa, the main reason is that a whitening effect due to myoglobin denaturation and/or haem displacement or release and oxidation of the ferrous myoglobin to ferric myoglobin above 400 MPa. Bolumar et al. (2012) showed that the beef colour changes caused by HPP were similar in appearance to the colour change upon cooking, such as lightness increased and redness decreased, although the colour changes induced by cooking and HPP have different mechanisms. The oxidation state of the iron in myoglobin is a key factor in the meat colour treatment by HPP. Because the light reflection and scattering increased, L^* value of pork increased at pressures up to 400 MPa. The other reason is possible that the myofibrillar proteins decreased the solubility and formed larger insoluble protein aggregates during the HPP, which might affect the meat surface and the light reflectance (Olsen & Orlien, 2016). Wackerbarth et al. (2009) found that the oxy-myoglobin was changed into the formation of met-myoglobin and further denatured ferric myoglobin species, the structural transition could cause a colour change and initiate unwanted oxidative side reactions involving further components of meat. HPP treated meat samples have a high ultimate pH, which led to their colour becoming darker. Brewer et al. (2001) reported that the higher pH of HPP treated was correlated with lower L^* values causing the meat to appear darker. The chicken meat has a low content of myoglobin, is considered "white meat". Therefor, the colour of the raw breast is slightly pinkish, the appearance is bluish-white to yellow. The lightness, redness and yellowness of whole chicken breast fillets were increased after 300-600 MPa treatment. The increase in redness was caused by the reversible renaturation of pressure-denatured myoglobin (Kruk et al., 2011; Olmo et al., 2010). Overall, HPP has a great impact on meat colour, which is an interrelationship of the modifications of myoglobin molecules.

Effects of HPP on the water holding capacity of muscle. The effects of HPP on the water holding capacity of meat depended on pressure levels, treatment time and temperature. Ma et al. (2019) reported that with increasing time and pressure, the water holding capacity of the yak meat increased first and then decreased. At 250 MPa, 15 min, the water holding capacity had increase of 10.50% and the meat turned white. The reason is that HPP is caused by reduced exposure of acidic groups and increased the pH levels of meat, which could improve the water holding capacity. When the HPP and time were exceeded, the meat has an excessive contraction and the water holding capacity decreased (Hong et al., 2005). Souza et al. (2011) also reported that the cooking loss of pork was decreased by 17.35% after being exposed to 215 MPa at 33 °C for 15 s. If the pressure lever is too high, the activity of calpain, such as desmin, is been inhibited by HPP and prevents the degradation of cytoskeletal proteins, and reduces the water holding capacity of muscle (Campus, 2010). The HPP and heat combined could improve the water retention in the muscle, depending on the process parameters. When pressure treatment at 100 to 200 MPa, the drip loss and free water of pork meat were increase from 4% to 7%, and at 300-400 MPa these was decreased to 4%. The cooking yield of pork meat by previously HPP treated from 300 to 400 MPa was significantly higher than the heated-only samples; they were no difference between heated-only and HPP treated samples (Korzeniowski et al., 1999). These different results for water holding capacity were caused by the different temperatures and pressure levels. Some researchers have reported that the HPP could be used to improve the quality of heterogeneous meat. The pale, soft, and exudative (PSE) meat has lower muscle pH and is associated with lower water holding capacity. The HPP may improve the water holding capacity of PSE meat. Chan et al. (2011) found that the expressible moisture of PSE-like turkey breast meat was decreased at 50 and 100 MPa, and the lowest level occurred at 100 MPa (18.7%), the result in that the water holding capacity was increased at these pressure levels. However, the water holding capacity was decreased significantly at 150 MPa for 5 min at 4 °C, because of the less hydrophobic interactions and lower protein surface hydrophobicity at 150 MPa. In addition, the effects of water characteristics in meat treated by HPP have been researched. Bertram et al. (2004) reported that the T_2 values were lower in pressure-heat treated meat revealing alterations in water characteristics of pressure-treated and the shear force of pressure treated samples were lower. The HPP has affected the myofibrillar organization, which changes the properties of water in the meat and improved its tenderness of meat (Bertram, Purslow, & Andersen, 2002).

The effects of HPP on the tenderness of muscle. Tenderness is identified as the primary eating quality factor, which is the key determinant of whether consumers are repeat buyers or not (Miller, Carr, Ramsey, Crockett, & Hoover, 2001; Platter et al., 2003). The tenderness depends on the myofibrillar and connective tissue proteins. The mechanisms of meat tenderisation that occurs in HPP of pre-rigor muscles and chill aging of post-rigor muscles are different. HPP could cause the changes in muscle microstructure, sarcomere contraction, muscle fiber damages, and myofibril fragmentation, such as hydrolyzed the proteins in the muscle fibers, weakening the cell structure, releasing the ions and activating calcium activating enzymes (Lowder et al., 2014). Calpains are a large family of cytoplasmic cysteine Ca^{2+} dependent proteases in skeletal muscle, they are in contact with the post-mortem proteolysis and meat tenderization, which are able to degrade myofibrillar proteins including nebulin, titin, troponin-T and desmin (Hufflonergan et al., 1996; Kristensen & Purslow, 2001). Homma et al. (1996) found that the calpain activity of muscle was increased by pressure up to 200 MPa caused by Ca^{2+}, which was released from the sarcoplasmic reticulum and the inactivation of the inhibitor calpastatin during the pressure treatment. Morton et al. (2018) reported that HPP could direct physical disruption of the sarcomeres, and destroyed the organised structure of the sarcomere Z discs, M lines and A bands. Bouton et al. (1977) obtained that HPP is a clean technology that can tenderise post-rigor meat with the appropriate pressure levels and temperatures. Souza et al. (2011) found that the shear force of pork was decreased by 30% after 215 MPa for 15 s at 33 °C. After 300 MPa for 20 min at 20 °C treatment, the shear force of the goose breast was decreased by 34.78% (Gao et al., 2014).

The shear force of hot-boned beef was decreased after 175 MPa treatment and improved the eating quality. Thus, the moderate pressure levels treatment of pre-rigor meat seems to have potential since the meat was tender and looked normal (Morton et al., 2017; Bonilauri et al., 2021). Ma and Ledward (2013) reported that the tenderness of pre-rigor meat after being subjected to pressures of about 100-150 MPa was significantly improved compared to the untreated counterpart, and this method has become a commercially viable process, given the decreasing cost of HPP machines. Up to 60 °C, the shear force of post-rigor meat was significantly reduced after being subjected to pressures of 100-200 MPa. Some authors have reported that the post-rigour beef muscle treatments by HPP had no beneficial effects, such as combined pressure-heating treatments, which result in brown discolouration (Ma & Ledward, 2004; Ma et al., 2007).

The connective tissue proteins are an important factor in the tenderization of meat, such as the state of linking myofibrils to the sarcolemma and other filaments from the cytoskeletal network (Cheftel & Culioli, 1997; Taylor et al., 1995). The thermal solubility of collagen was changed caused by 200-500 MPa at 20 °C for 10 min, the thermal stability of thermally undenatured collagen was improved, and the thermal stability of partial collagen denaturation before pressurization might be reduced. Ichinoseki et al. (2007) found that the thermal stability and surface hydrophobicity of beef collagen fibrils was decreased during treatment by HPP, caused the structural weakening of intramuscular connective tissue. The intramuscular connective tissue was benefit of improving tenderness. Kim et al. (2007) showed that the shear force of the bovine M. *semitendinosus* muscle was decreased significantly treated by 100-500 MPa at 15 °C for 5 min, after cooking to an internal temperature of 75 °C.

1.1.3 Effect of HPP on the comminuted meat products

The traditional comminuted meat products contain higher salt and fat, overtaking the salt and fat could increase the risk of obesity, hypertension and cardiovascular disease (Kang et al., 2017; Delgado-Pando et al., 2010; 2015; Jeon et al., 2015; Yalcin & Seker, 2016). However, the salt and fat content have a key factor in the solubilization of the myofibrillar proteins, because these proteins determine the binding and textural characteristics of the products, they are also contributes to the flavor of comminuted meat products (Pietrasik & Li-Chan, 2002; Tobin, O'Sullivan, Hamill, & Kerry, 2013; Bernasconi et al., 2020). For declining the animal salt and fat contents, HPP has caught the interest of emulsion meat products because it meets consumer's requirements for low fat and salt content, which has been renewed as the best non-thermal intervention for extending the shelf-life and safety of comminuted meat products without altering sensory and nutritional properties (Hygreeva & Pandey, 2017; Chen et al., 2018).

Effects of HPP on the water and fat holding capacity of comminuted meat products. The water and fat holding capacity express the ability of comminuted meat product to hold water and fat, which is an important indicator of products quality. Proteins undergo unfolding and denaturation followed by protein association, forming a three dimensional network that entraps water molecules and thus produces a gel. The pressure intensity, salt content, meat type, composition, temperature and others factors independently affected both the water and fat holding capacity of comminuted meat products. Carballo et al. (2000) found that the post-rigor pork gel structures had better water binding properties but were weaker than non-pressurized meat batters and batters pressurized prior to heating. Zheng et al. (2017) reported that the cooking loss was not decreased caused by the addition of salt with HPP treated chicken meat batters, this suggested that HPP was much more effective than salt in reducing water loss during the cooking. Rospolski et al. (2015) reported that water became slightly more tightly bound to the meat matrix after HPP, main reason is that HPP increase the solubility of muscle proteins, thus increasing water and fat holding capacity and decreasing mechanical water loss (Chan et al., 2011; Sikes, Tobin, & Tume, 2009). Villamonte et al. (2013a and b) also observed an increase in water holding capacity due to the interaction of HPP and salt in pork batter, this maybe because increasing sodium chloride causes increasing denaturation of muscle proteins in HPP treated meat batters and favors the solubilization of proteins and the formation of a gel network.

The HPP and heating (> 40 °C) combinations limit the gelling process of meat systems. The pork and chicken batters had better water binding properties after 200–400 MPa treated for 30 min, at 60-80 °C, however, the gel structures were weaker than gels made by only-heating or pressurized prior to heating (Fernandez, 1998; Colmenero, 2002; Yang et al., 2015). Marcos et al. (2010) also reported that a higher cooking yield was observed at 40 °C compared to 60 °C in ostrich meat sausage by pressurization before heating. Under adequate conditions, application of HPP modifies the functionality of non-meat protein and polysaccharide molecules and significantly promotes the emulsifying activities and stability. Moreover, some researchers have reported a synergistic effect of dietary fibre, soy protein isolate, starch, hydrophilic colloid, and other materials and HPP combined on water and fat holding capacity in HPP treated comminuted meat products. Grossi et al. (2011) reported that HPP and carrot dietary fiber markedly improved emulsion strength resulting in firm pork sausages. Moller et al. (2011) found that the significant effects of pressure temperature, holding time, and addition of carrot fiber on the distribution and mobility of water, and the T_2 relaxation times were able to explain more than 90% of the variation in water holding capacity for both non-pressure and pressure-treated sausages, combined HPP and addition of fiber caused non-coherent changes in T_2 NMR relaxation times. Chun et al. (2014) showed that the addition of binders, such as soy protein isolated, wheat flour, and κ-carrageenan, improved water-binding properties of pressure or non-pressure-induced restructured pork, but lowered the hardness. Hong et al. (2004) found that HPP and added isolated soy protein, sodium caseinate, whey protein concentrate and egg white powder improved the water binding capacity and binding strength of the restructured pork, respectively. However, due to the excessive protein damage reflected as increased surface hydrophobicity, less protein-water interactions and thus lower water-binding properties of sodium caseinate and whey protein concentrate, added sodium caseinate and whey protein concentrate were not effect on water binding properties under HPP. Thus, the application of HPP had more effects on restructuring meat than binders (Uresti et al., 2004). Trespalacios and Pla (2009) used the dried egg white as a fat replacement to obtain a low-fat chicken gel by means of HPP, the water binding properties and hardness were improved, suggesting their participation in the network structure coupled to the myofibrillar proteins, and noted that the modifying certain functional characteristics of chicken gels with low fat content by means of HPP and the addition of dried egg white.

Effects of HPP on the texture of comminuted meat products. The texture of comminuted meat products is an important factor to determined the consumers purchase or not. HPP induced texture modifications have been used to affect myofibrillar proteins and their gel-forming properties, raising the possibility of the development of processed comminuted meat products. Over 200 MPa treatment, the protein extractability was decreased significantly in meat batters, and caused protein denaturation and/or aggregation, which limited their functionalities (Oflynn et al., 2014a, b; Sazonova et al., 2019). The M-line and Z-line of the chicken myofibril in 0.2 M NaCl were disrupted, and the thin and thick filaments were dissociated by HPP. The microstructure of pressure–heat-induced chicken myofibrillar gel was composed of three-dimensional fine strands. Pressurization, at 200 MPa, prior to heating, increased the apparent elasticities of chicken myofibrillar gel; however, pressure treatment above 200 MPa decreased it (Iwasaki et al., 2006). Yang et al. (2015) found that the textural properties of hardness, chewiness, springiness, cohesiveness and resilience were significantly increased at an interval of 100 MPa and 200 MPa, except for the textural property of adhesiveness up to 200 MPa, but no changes of hardness, chewiness, springiness and resilience were observed up to 300 MPa and 400 MPa. Hwang, Lai, and Hsu (2007) showed that the sausages had a harder texture after 200 MPa, because partly depolymerized, unfolded, aggregated and denatured the extracted proteins under 200 MPa, which caused the changes in water distributions, formed new protein components, and solubilization or denaturation of myofibrillar proteins. Hygreeva et al. (2016) studied the effects on quality characteristics of precooked chicken patties were subjected to HPP at 200, 400 and 600 MPa for 10 min, the result indicated that the textural properties of chicken patties were improved after being treated at 200 and 400 MPa. Crehan et al. (2000) found that the texture of frankfurters were improved after 150 and 300 MPa treatment at low salt content (1.5%). Therefore, the texture properties of comminuted meat products with low slat content could be improved at moderate pressure levels (100-300 MPa).

The changes of texture properties also are related to the pressure temperature and processing, the possibly is that the modified conformation, slowed heat-denaturation, together with disrupted myofibrillar eventually led to the different batter structures. The cooking of meat batters either before or after HPP results in varying effects on meat product texture. Mor-Mur and Yuste (2003) found that the textural properties of vacuum packed cooked sausages were treated at 500 MPa and 65 °C, 5 or 15 min improved cohesiveness and increased fracture force of the product. Zheng et al. (2017) showed that the physical properties of batters were subjected to HPP (0-400 MPa, 75°C, 30 min) depending on the pressure intensity. The chicken meat batters treated at 200 MPa exhibited desirable qualities, having a smooth appearance and rigid texture, while those treated at 400 MPa had undesirable qualities, being coarse and watery in appearance, with a weak texture. The structural changes induced in proteins of meat batters were partially reversible at low temperatures when increasing the pressure from 100 to 300 MPa, however, these changes were irreversible when the pressures were beyond 300 MPa (Rastogi, Raghavarao, Balasubramaniam, Niranjan, & Knorr, 2007). Carballo et al. (2000) reported that the pork batters treated by HPP prior to heating decreased the hardness, springiness and chewiness, formed a coarse, irregular, loose protein matrix and favoring weaker gel structure, because HPP limits protein-protein interaction (Carballo, FernaÂndez & JimeÂnez-Colmenero, 1996).

Some researchers had reported that HPP induced muscle protein gels form a firmer texture. Yang et al. (2016) reported that compared with the values of 0.1 MPa treated sausages, the 200 MPa for 2 min at 10 °C was significant increased in all the textural values. Zheng et al. (2015) showed that HPP before heating sausages had significantly higher values for hardness, springiness, cohesiveness, chewiness and resilience than did the only-heat sausages. It is well known that HPP causes protein denaturation with increasing the pressure and temperature (Tintchev et al., 2013; Rastogi et al., 2007). The myosin protein have completely denaturation by 200 MPa, at 50 and 60 °C, the reason is the pressure and heat combined could be improved the efficiency of protein aggregation and gelation, and form a heat induced helix-coil transition (Buckow, Sikes & Tume, 2013). This indicated that excessive temperature resulted in the weakening of molecular interactions and the destruction of the network structure in gels (Colmenero, 2002). In addition, the temperature with HPP was affected the state of moisture in the pork batters, which in turn affected the texture of the gel (Cando et al., 2014). Other factors also affected the texture properties, such as added non-meat proteins, hydrophilic colloid. Hong and others (2008) found that the breaking force and tensile strength of restructured pork meat treated by 200 MPa combined with κ-carrageenan were increased, and the pressure above 200 MPa and the addition of 1.5% κ-carrageenan has potential use in cold-set meat restructuring. Grossi et al. (2012) reported that the use of carrot fiber and potato starch had more impact on textural properties in pork sausages with low salt content (1.2%) treated by HPP, and water binding capacity of low salt pork sausages was improved, which produced sausages with better sensory properties. Lee et al. (2018) indicated that due to the pH and protein solubility were increased after being subjected to HPP, the water holding capacity and instrumental hardness of sausages treated with a combination of sea tangle powder and HPP were similar to the sausages with 0.2% sodium pyrophosphate, and greater inhibition ability against lipid oxidation and bacterial growth.

1.1.4 Effect of HPP on the gel properties of myofibrillar proteins

Myofibrillar proteins account for 50%~55% of the total protein content in muscle, mainly composed of myosin and actin. Myofibrillar proteins are salt-soluble proteins, which are soluble in high ionic strength solution (> 0.3 M), it is decided to the gel properties, such as water holding capacity, texture, shelf life. During the gel form, the helix-coil transitions of myosin tails and subsequent aggregation of myosin heads through intra- and intermolecular interaction, and then a three dimensional and crosslinked network is formed after partial unfolding or denaturation of myofibrillar proteins. There is no doubt that HPP induces certain alterations in myofibrillar proteins which influence their functional properties. The HPP is able to variable alteration-200 MPa, the tertiary structure is significantly affected above 200 MPa, and secondary structure changes take place at 300-700 MPa, which could improve its gelation properties.

Effects of HPP on the water holding capacity of myofibrillar proteins. The water holding capacity of myofibrillar proteins was affected by the level, time and temperature of HPP. When the pressure levels were low (≤ 200 MPa), which could improve the solubility of myofibrillar proteins; over 300 MPa, which could reduce solubility of myofibrillar proteins and form the large aggregates observed that the denaturation of myosin of bovine occurred owing to the release of myosin light chain at 200 MPa, and the rate of myosin denaturation increased rapidly at pressures above 300 MPa because of the aggregation of myosin heavy chain. Actin was released at 200 MPa and the denaturation of actin might have been accelerated by the aggregation of released actin at pressures above 300 MPa. The solubilization of myofibrillar proteins also was affected by temperature during HPP. The solubilization of myofibrillar proteins increased with increasing temperature, especially from 40 °C to 60 °C, and a regular trend of protein solubilization was found when isolated myofibrils were subjected to HPP at different temperatures, an increase was observed with increasing pressure up to about 400 MPa, solubility then decreasing to 600 MPa. Barriosperalta et al. (2012) found that myofibrillar proteins from abalone and starch interaction increase the emulsifying capacity at pressures over 350 MPa applied for 3-5 min, myofibrillar proteins and egg white interactions at pressures higher than 450 MPa for 5-10 min formed coagulation, decreasing the emulsifying capacity. The reason is that myosin of pressure-induced surimi gelation denaturation and concomitant disulfide bond formation at 300 MPa, 5 °C for 30 min. During heating the pork myofibrillar proteins, aggregation of meat proteins caused the meat protein matrix to shrink, which reduced the amount of water that could be bound by the matrix, causing the cooking loss to increase. Yang et al. (2015) found that HPP (200 MPa for 2 min) significantly decreased the cooked loss of reduced-fat and reduced-salt pork sausages, and changed the P2 peak ratio of the four water components in raw pork sausages. Therefore, the HPP has an important commercial and health benefit of the altered properties of myofibrillar proteins, which is their ability to form gels that have very high cook yields even in the presence of low salt. Zhang et al. (2015) showed that the myofibrillar proteins of chicken breast meat were treated at 100, 200, 300, 400, 500 MPa and kept for 10 min, the centrifugation loss increased gradually from 36.59% (0.1 MPa) to 37.28% (200 MPa) and decreased sharply from to 30.82% (300 MPa), then decreased slowly to 30.12% (500 MPa); the relaxation time of T_{2b} decreased from 2.31 to 1.32 ms, T_{21} had no significant changes, and T_{22} increased from 2477.08 to 3274.55 ms, that means bound water had lower water mobility, immobilized water had no significant changes and free water had a higher water mobility.

Effects of HPP on the texture of myofibrillar proteins. The texture is an important characteristic of myofibrillar proteins gel, which decides the quality of meat products. The solubility of myofibrillar proteins affects the texture of myofibrillar proteins gel, because the functional properties require the solubilization of the proteins. HPP is an important thermodynamic parameter that can profoundly influence molecular systems, it induces the depolymerisation of myofibrillar proteins with a consequence of increasing solubility. Iwasaki et al. (2006) found that the elasticity of chicken myofibrillar gels were apparent increased by 2- or 3-fold at 200 MPa (10–20min), prior to heating at 70 °C. Cando et al. (2015) showed that the surimi gel had a higher breaking force after 150 MPa treatment, but decreased the breaking force after 300 MPa treatment. It is well known that HPP is an important thermodynamic parameter that can profoundly influence molecular systems. When the HPP over 400 MPa can readily denature proteins, and 200 MPa only affects their quaternary structures, leading to the dissociation of oligomeric proteins. Zhang et al. (2017) reported that the gel hardness of myofibrillar proteins increased from 20.25 (0.1 MPa) to 46.6 g (200 MPa), then decreased gradually to 33.3 g (500 MPa). The main reason is the HPP could affect molecular interactions and protein conformations, which lead to myofibrillar protein's denaturation, dissociation, aggregation, then resulting in modified functional properties. Angsupanich et al. (1999) found when isolated myofibrillar protein from turkey was pressure treated at 200 MPa, there was no change in any of the peaks of DSC, up to 400 MPa and above caused loss of the myosin peak and major loss of actin structure and a 'new' peak. Ko et al. (2003) reported that the increase of the surface hydrophobicity of myosin with improving the pressure levels, which caused the structural changes of myosin, would compensate for the decrease in the gel strength of myosin, this would cause the decreases in G′ values.

Textural properties of protein gel greatly depended on its microstructure. Ma et al. (2011) reported that myosin light chains and actin thin filaments of beef muscle were sensitive to pressure, they were released from myofibrils subjected to 100 MPa. Suzuki et al. (1991) found that the proteins of actin, tropomyosin, troponin C as well as M-protein were solubilized at 100 MPa, whereas solubilization of myosin heavy chains over 300 MPa. Therefore, the muscle type, pH, temperature, and salt type and concentration affected the solubility and texture during the HPP. Cao et al. (2012) observed by scanning electron microscopy that the network structure of rabbit myosin thermally induced gel was small and uniform after 200 MPa, while the gel holes became larger above 200 MPa, and the G′ and G″ values were decreased with the pressure levels increased. Zhang et al. (2017)

found that due to the myofibrillar proteins were partial unfolded, the gels contained many filaments and irregular cavities at 100 MPa; the smallest particle size of myofibrillar proteins was formed at 200 MPa, the gels had a denser and homogeneous network, and the hardness had the largest value; the myofibrillar proteins denatured excessively, interior hydrophobic and sulfhydryl groups exposed above 300 MPa, the gel cavities became larger and heterogeneous, and the hardness was decreased. Overall, myofibrillar proteins gels with higher hardness had smaller, denser and homogeneous gel microstructure, while gels with lower harness had larger cavities and coarse microstructure.

Effects of HPP on the protein conformation of myofibrillar proteins. HPP can affect myofibrillar protein's molecular interactions and protein conformation, leading to protein denaturation, aggregation, or gelation that presents altered functional properties. Which could be to improve the gel-forming properties of muscle proteins, a crucial factor in processed muscle-based food. The pressure induced aggregation involved the dissociation of myosin heavy and light chains followed by aggregation of the heavy chains. The proteins from the thin filament such as actin, tropomyosin, troponin C as well as M-protein were solubilized at 100 MPa, and myosin light chains also were sensitive to pressure, and were released from myofibrils subjected to 100 MPa, whereas solubilization of myosin heavy chains required up to 300 MPa. Some authors had reported that the HPP affects chemical forces of myofibrillar proteins. Due to more tryptophan hydrophobic residues and phenolic hydroxyl groups of tyrosine residues tended to be buried in a hydrophobic microenvironment and generated hydrogen bonds with protein molecules, the hydrogen bonds appeared to be strengthened under pressure. The intermolecular H-bonds between proteins were formed and caused the aggregation, which could decrease the solubility of myofibrillar proteins when the pressure up to 400 MPa and above, due to the protein-protein interaction at pressure of 400 MPa is formed at the expense of protein-water interactions, and the intermolecular H-bonds between proteins are stronger than the H-bonds between protein and water (Bai et al., 2021). Angsupanich et al. (1999) studied the effect of isolated myofibrillar protein and myosin of cod or turkey (pH $\approx$ 7) were subjected to pressures up to 800 MPa for 20 min, and found that HPP-induced denaturation of myosin led to the formation of structures that contained hydrogen bonds and were additionally stabilized by disulfide bonds. It is well known that the breakdown of a disulphide bond requires energy of 213.1 kJ/mol, but the HPP at 10000 MPa only provides only 8.37 kJ/mol. Thus, the increase in the reactive sulphydryl group content might cause by a change of myosin structure involving the active sites of myosin, which could lead to changes in actomyosin formation and enzymatic properties of myosin. The surface hydrophobicity was significantly positive with pressure level.

The myofibrillar proteins became more unfolded with the pressure increased, more buried hydrophobic residues were exposed, and more hydrophobic sites or pockets of protein molecules could bind to the ANS (1-anilinonaphthalene-8-sulphonic acid), then large protein aggregates were formed. Zhang et al. (2015) found the surface hydrophobicity of myofibrillar proteins from chicken breast meat increased slowly from 0.1 to 100 MPa, and then a sharp increase when treated by HPP above 200 MPa. Cao et al. (2012) showed a clear positive relationship between pressure level applied and hydrophobicity, and increased significantly above 200 MPa, which means an increased denaturation and unfolding of myosin and greater exposure of amino acid residues with increased pressure. Chapleau and de Lamballerie-Anton (2003) studied the effect of pressure (0-600 MPa) and time (0-1800 s) on the surface hydrophobicity, and reactive sulphydryl groups content of bovine myofibrillar proteins in solution at 10 g/L, the results found that HPP induced a threefold increase of the surface hydrophobicity of myofibrillar proteins between 0 MPa and 450 MPa. The same upward trend was obtained on the reactive sulphydryl groups, which increased from 40% to 69%. The increasing linked with the change of the secondary structure and the destruction of the α-helices present in the heavy chains of myosin. Due to 100 or 200 MPa is too low to affect the exposure of buried sulfhydryl groups, the SH content of myosin was not significantly differences, at 300 MPa and above, the SH content was significantly increased, the increase of sulfhydryl groups might be explained by the change of myosin structure.

The secondary structures of meat protein are sensitive to changes in the hydrogen bonding scheme involving the peptide linkages of the amide I band, which is attributable to α-helice, β-sheet, β-turn and random coil structures, respectively. Berhe et al. (2014) reported that the meat protein cooked above 60 °C was positively correlated to the high intensity of bands at the amide I regions. The results indicated that it was a significant increase in the β-sheet and β-turn structure content accompanied by a concomitant decrease in α-helice content. Zhang et al. (2017) found that increased the pressure levels, α-helix and β-sheet changed into random coil and β-turn, and the surface hydrophobicity and formation of disulfide bonds were strengthened. Compared with the only-heat, when HPP at 200 MPa, 15 min, the contents of β-sheet and β-turn were significant increase from 20 °C to 40 °C, and there was no significant different from 50 °C to 60 °C, because of the myofibrillar protein had completely denaturation. The HPP was significantly effected of the gel properties and protein conformation of myofibrillar proteins gel. The HPP could provide great potential for myofibrillar protein structural modification, such as leading to protein denaturation, solubilization, aggregation or gelation, thereby creating innovative functional properties. A moderate pressure (< 200 MPa) can enhance the water holding capacity and texture of myofibrillar proteins gel.

It is well established that HPP will improve the properties of muscle, comminuted meat and myofibrillar proteins. The use of moderate pressure treatment of pre-rigor meat seems to have potential since the meat will be tender and look normal colour. Reasonable HPP could enhance the water holding capacity and texture of comminuted meat, but the products lacked the cooked appearance and potential for accelerated loss of flavour. Which also affected the non-covalent bond, covalent bond and protein conformation of myofibrillar proteins, the water holding capacity and texture of myofibrillar proteins will be increased and produced by moderate pressure treatment. However, the affecting factors on properties of muscle, comminuted meat and myofibrillar proteins by HPP are complex, still need a lot of research in the future.

1.1.5 Application of HPP in reduced-salt gel meat products

The historical experience of salt reduction showed that reducing the per capita sodium chloride intake requires the effective participation of the food processing industry (Aliño, Grau, Fuentes & Barat, 2010; Paula et al., 2019; Li et al., 2021), among which the most effective method was adopted new processing technology to reduce the sodium chloride content in meat products. According to the traditional thermal gel mechanism of meat proteins, sufficient sodium chloride can extract the salt-soluble protein, such as myosin and actin, to form good texture and taste. The way of directly reducing sodium chloride could cause the product yield and edible quality to be significantly reduced (Barbut & Mittal, 1988; Desmond, 2006; Li, Zhang, Lu & Kang, 2021). Therefore, how to reduce sodium chloride content while ensuring the good quality of meat products has become an urgent problem to be solved in the meat industry.

The effects of sodium chloride in gel meat products. Sodium chloride plays an important role in gel meat products. First, sodium ions and chloride ions can stimulate taste. Second, myofibrillar protein can be extracted to facilitate the dissolution and swelling, which increased the water- and oil-retaining properties of the gel, and improved the product yield, texture and shelf life (Kang et al., 2014; Alvarez et al., 2007, 2012; Yao, Zhou, Chen, Ma, Li & Chen, 2017; Kang, Hu, Zhu & Ma, 2018; Lu, Kang, Wei & Li, 2021). The function of dissolving and extracting myofibrillar was called the processing effect of sodium chloride, which was the key to forming the quality of gel meat products (Desmond, 2006; Mancini, Nuvoloni, Pedonese & Paci, 2019). The swelling of myofibrillar was also very important for the processing of meat products, it was wrapped around the meat and fat particles or liquid drops. During the heating, the substances were cross-linked and the water was trapped in the protein matrix. Offer and Knight (1983) reported that sodium ions can form the electron clouds around myofibrillar molecules and promote the dissolution of myofibrillar protein. In fact, the sodium chloride from 1% to 1.5% can meet the majority of consumers' demand for salty taste, however, to meet the processing required, the gel meat products generally add 2% to 4% sodium chloride (Hand, Terrell, Zhou & Smith, 1982).

The biggest obstacle to reducing sodium chloride in meat products is that sodium chloride is a very cheap ingredient, and consumers are more comfortable with the quality and flavor of meat products with adding sodium chloride. On the premise that consumers can accept, the main ways to reduce the sodium content of gel meat products are summarized as follows: reduced the amount of sodium chloride added and replaced it with other salts; the *Glutamine transaminase* was added to catalyze the interprotein (or internal) acyl transfer reaction to form covalent cross-linking between proteins (or polypeptides) (Colmenero, Ayo & Carballo, 2005; Kang, Li & Ma, 2017). The new process can improve the performance of myofibrillar, and still form a good gel at low ionic strength (Desmond, 2006; Kang, Li, Ma & Chen, 2016; Inguglia, Zhang, Tiwari, Kerry & Burgess, 2017).

The overall use of salt substitutes was difficult for consumers to accept, but the partial reduction of salt in meat products was a desirable approach. There were many kinds of salt, but few of them can be used to process gel meat products successfully or completely instead of sodium chloride (Inguglia, Zhang, Tiwari, Kerry & Burgess, 2017; Mariutti & Bragagnolo, 2017). In most studies, sodium chloride was replaced by other chloride salts, because the processing effect of sodium chloride was mainly achieved through the binding of chloride ions with proteins. The decrease in chloride ion content was led to a significant decline in gel properties. At present, the most successful alternative salt in the study is potassium chloride, which can replace about 35%~40% sodium chloride in the formula of gel meat products, but excessive potassium chloride produced bitter and other bad smells (Zhang, Wu, Jamali, Guo & Peng, 2017). Polyphosphate can increase the pH of meat products, cause muscle fibrils to swell, facilitate actomyosin dissociation, while it can partially replace sodium chloride. Reducing sodium chloride crystal size and changing crystal shape can reduce the amount of sodium chloride addition without affecting food saltiness. However, gel meat products contained a lot of water, and sodium chloride was dissolved in water, so the effect of reducing the content of sodium chloride in gel meat products, thus, this method is limited (Angus et al., 2006).

Another way was to use flavor enhancers. Flavor enhancers increased the saltiness and flavor of reduced-salt meat products, it decreased the use of sodium chloride without reducing the saltiness and flavor of meat products. Some flavor enhancers and shaders had been used in industrial production, and the usage was increasing, such the products include yeast extract, lactate, sodium glutamate and nucleotide. Flavor enhancers can stimulate the taste and reduce the stimulation of sodium chloride to the taste nerve, helping to reduce the amount of sodium chloride used. Pasin et al. (1989) used potassium chloride and nucleotide mixture (50% IMP and GMP mixture used commercially) to reduce sodium chloride in pork sausages by 75%. Any amount of glutamate in these pork sausages combined with potassium chloride can be substituted for 50% salt. Other compound flavor enhancers, such as lysine and succinic acid mixtures, had been studied as substitutes for salt. This kind of complex has the flavor of salt, as well as antibacterial and antioxidant properties, it can replace 75% of salt and has a good development prospect (Triki et al., 2017). Through adding phosphate, starch and hydrocolloid to make up for decreasing water retention and product quality of reduced-salt meat products, the use of different levels of potassium lactate or sodium lactate as an alternative salt to maintain the product's flavor and saltiness (Omana, Plastow & Betti, 2011).

The use of HPP in reduced-salt meat products. The HPP improved the functional properties of meat protein, and was beneficial for reducing salt (Duranton, Guillou, Simonin, Chéret & De Lamballerie, 2012; Chen et al., 2018; Zheng et al., 2017). Such as, the surface hydrophobicity and total sulfhydryl groups of rabbit myosin were increased under HPP between 100 and 200 MPa (Chapleau et al., 2004). Meat protein was sensitive to the HPP. The α-helix and β-sheet structures changed into random coil and β-turn structures as the pressure levels increased; moreover, the protein solubility and gel hardness reached their maximum values and the gel microstructure was dense and uniform at 200 MPa. Thus, a better understanding of the changes in gel properties and protein conformations occurring in meat products induced by combined HPP and thermal conditions could be helpful to elucidate their role during gel formation, and facilitate the development of new healthy meat products (Zhang et al., 2017; Yang et al., 2021). Sensory evaluation was conducted after the HPP of reduced-salt frankfurter by HPP, and it was found that the tasters were more likely to accept the sausages treated with reduced-salt and HPP, which indicated that HPP improved the texture of the sausages, and partially reduced the amount of sodium chloride (Crehan, Troy & Buckley, 2000). Grossi et al. (2011) reported that the use of HPP technology reduced the sodium chloride content from 1.8% to 1.2% of pork sausage with carrot fiber and potato starch, it had no negative impact on water-retaining performance, colour and texture. Increased the pressure and temperature, the meat batter with carrot fiber formed a highly elastic, organically combined and orderly network structure (Sun, Wu, Xu & Li, 2012).

Effects of HPP and heat combination in reduced-salt meat products. It is well known that the mechanism of meat protein denaturation and formed gel caused by heat and HPP were different. The HPP induced meat gels were based on the protein volume decline, while the thermal meat gels were caused by the violent movement of molecules and destruction of non-covalent bonds. Some researchers had reported the effects of reduced-salt meat products on the combination of heat and HPP (Khan et al., 2014; Tintchev et al., 2013; Chen et al., 2018; Zheng et al., 2017; Zheng et al., 2019). The temperature during HPP also affected water and fat holding capacity, and gel properties. Combining HPP and heat treatment at reduced-salt meat protein denaturation temperatures in a single-step process had reportedly resulted in better water retention and texture than in heat-only samples (Jimenezcolmenero, Fernandez, Carballo & Fernandezmartin, 1998; Zheng et al., 2015). HPP prior to thermal processing improved the functionality of meat batters. Wei et al. (2019) studied the effects of protein conformations and gel characteristics of reduced-salt (1% sodium chloride) pork batters produced by HPP prior to heating (20-60 °C), and who found that the highest cooking yield, hardness, springiness, chewiness, and G' values were observed in batters made by HPP at 20 °C and 30 °C. Meanwhile, the α-helix structure was significantly decreased, and accompanied by the increase of β-sheet, β-turn, and random coil structures at 20-40 °C. The reason is possible that the maximal solubilization of myofibrillar protein occurred at 200 MPa, with a reduction of salt content by 50% and improvement of functional properties, such as water-holding capacity and texture (Tintchev et al., 2013). Zheng et al. (2019) found that the HPP, rather than salt, was the main factor affecting the quality of chicken meat batter, the quality of reduced-salt chicken batter was improved by heating under 200 MPa and formed a fibrous network inside muscle fibers; meanwhile, application of HPP at a specified pressure was an excellent process for producing reduced-salt comminuted meat products, but excessive HPP resulted in inferior quality.

1.2 The effect on techno-functional properties of soy proteins by HPP

1.2.1 Functional properties of soy protein

The protein content of soy protein isolate is more than 90%, it is a high-quality plant protein food raw material. It's functional properties can be divided into three categories: interface properties, mainly including emulsification and foaming properties; hydration properties, including wettability, dispersability, solubility, viscosity and water retention;

properties related to protein-protein interactions, including precipitation, aggregation, and gel properties. Through hydrophobic interaction, electrostatic interaction, a hydrogen bond or disulfide bonds cross-linking, the spatial network structure is formed (Wang, Lin, Cheng, Wang & Tan, 2020; Cao, Fu & He, 2007; Ou, Wang, Tang, Huang & Jackson, 2005; Rhim, Gennadios, Handa, Weller & Hanna, 2000). On this basis, hundreds of countries in the world have developed thousands of food products containing soy protein in recent years. Soy protein and its modified products are widely used in meat products, protein drinks, dairy products, baked products and other foods due to their prominent functional properties. It plays an important role in supplementing protein, supplementing the nutrition of multiple types of protein, reducing the intake of animal protein, and giving food health care functions. Therefore, the functional properties of proteins are very important to food manufacturing and processing, they directly affect the quality.

1.2.2 Components of soy protein

Soy protein is mainly composed of β-conglycinin (7S globulin) and globulin (11S), accounting for over 70% of the total protein content (Tang, Wu, Chen & Yang, 2006; Utsumi & Kinsella, 1985). The 7S globulin is a trimer formed by the different combinations of three subunits (α', α and β), which are bound by hydrophobic and hydrogen bonds. The molecular weights of α', α and β are 65 kDa, 62 kDa and 57 kDa, respectively. Each 7S globulin contains a small number of disulfide bonds and is free of sulfhydryl groups (Saio, Watanabe & Kaji, 2006). The 11S consists of six subunits, its weight is 340-375 kDa. Each of these consists of an acidic polypeptide chain (A) and an alkaline polypeptide chain (B) connected by a disulfide bond to form the AB subunit. The 11S molecule contains more disulfide bonds and sulfhydryl groups (Matsudomi, Mori, Kato & Kobayashi, 1985; Liu et al., 2007). The differences in structure between 7S and 11S was affected on the formation of gel. Some studies have reported that 11S has better gel properties than that of 7S, but the emulsion capacity of 11S is lower (Saio & Watanabe, 2010; Saio, Kamiya & Watanabe, 2014; Pang, Safdar, Wang, Sun & Liu, 2020).

The functional properties of soy protein were affected by the concentration, temperature, pH, and so on (Ringgenberg, Alexander & Corredig, 2013; Schuldt, Raak, Jaros & Rohm, 2014). Such as, soy protein isolate concentration is one of the decisive factors in gel formation. The formation of soy protein isolate gel is the result of protein-protein and protein-solvent interactions, and the balance of attractive and repulsive forces between adjacent peptide chains. When the soy protein isolate concentration is low, protein-solvent interaction dominates, making it difficult for the system to form a gel (Wu, Ma & Hua, 2019). Therefore, the gel strength is positively correlated with soy protein isolate concentration. However, when the soy protein isolate concentration is lower than 8.0%, the gel cannot be formed only by heating. However, if the formation concentration of soy protein isolate gel can be changed to a certain extent by adjusting pH value, ion strength or modification, etc (Braga, Azevedo, Marques, Menossi & Cunha, 2006; Sirison et al., 2020). The other, pH and salt addition changed the ionization of functional groups of soy protein isolate and double electric layer thickness, affecting the protein-protein interaction (Wu, Navicha, Hua, Chen, Kong & Zhang, 2018; Opazo-Navarrete, Altenburg, Boom & Janssen, 2018). The salt concentration and type have different effects on the gel properties of soy protein isolate. At low ionic strength, salt can reduce the electrostatic repulsion between protein molecules by shielding the charge on the protein, and strengthening the gel strength. With the increase of ionic strength, the charge on the protein tends to be saturated, and the properties of water in the solvent change due to the presence of salt, leading to the enhancement of hydrophobic interaction, which becomes the dominant effect, and the gel strength decreases (Wu, Hua, Chen, Kong & Zhang, 2017; Puppo & Aón, 1998; Xia, & Abdalhai, 2015). Renkema, Gruppen and Van (2002) found that the denaturation of soy protein isolate occurred under all conditions of pH and ionic strength, such as a low stiffness gel was formed when pH > 6.0, on the contrary, a high stiffness was formed when pH = 5.0. Meanwhile, extensive rearrangements in the network structure took place during prolonged heating when pH = 7.6, whereas at pH 3.8 rearrangements did not occur.

1.2.3 The effects of soy protein by HPP

The globulin of soy protein has a closely globular structure, the molecular weight is small, and active group packages within the molecule, and some methods of modification are difficult to effectively change its structure, improve its functional characteristics. Thus, the function of 11S globulin is worse than the 7S globulin, it is a key factor that restricts the application of soy protein isolate and modification. How to effectively improve the structure of soy protein isolate becomes the primary factor of soy protein modification (Zadeh, O'Keefe, Kim & Cho, 2018; Guo, Lin, He & Zheng, 2020).

The changes of protein spatial structure caused by HPP are the focus of current research. In general, HPP has no effect on the primary structure of proteins, it has some effect on the secondary structure, and has a great effect on the tertiary and quaternary structures. The effect of HPP on protein can be reversible or irreversible. Generally, protein changes are reversible under 100-200 MPa. When the pressure exceeds 300 MPa, protein changes tend to be irreversible, that is, protein permanent denaturation (Balny & Masson 1993; Mozhaev, Heremans, Frank, Masson & Balny, 1996 and 2015; Heremans & Smeller, 1998). In food applications, the various functional properties of soy protein are mainly realized by the physical and chemical properties of storage proteins, namely, 7S and 11S globulin, which are ultimately determined by the intrinsic physical and chemical properties of proteins based on their molecular structure. Li, Zeng and Peng (1999) found that the change in molecular structure of soy protein isolate after HPP was caused the change of its related physical and chemical properties. The solubility of protein in low concentration (4.0%-4.5%) soy protein isolate solution was significantly improved after HPP, leading to the apparent viscosity were increased with the increase of pressures, and the values of G' and G" were proportional to the apparent viscosity. In a constant pressure force (400 MPa) or less under the action of HPP, 11S globular depolymerization of soy protein, protein molecules depolymerized to smaller particles on the unit, and the base unit of a certain degree of stretch further, makes the globular protein within the exposed polar groups and hydrophobic groups, and makes the protein molecules (particles) to strengthen the surface charge of distribution, then the combined water around the newly exposed polar groups were increased. Su, Li, Zhao, Liu & Zhang (2009) found that HPP (200-600 MPa) could change the large particles to smaller, the volume fraction of soy protein isolate occupied in the solution was significantly increased, making the dispersibility of soy protein isolate obviously improved. Meanwhile, the free sulfhydryl content and surface hydrophobicity (H_o) of soy protein isolate were significantly increased at pressure treatment of 400-600 MPa for 20 min, and the results of SDS-PAGE indicated that the subunits composition of soy protein isolate was greatly changed, which caused the content of 7S and 11S protein was obviously increased.

The pressure level and other factors (time, temperature) were influenced of the properties of soy protein. Zhang, Li, Tatsumi and Isobe (2005) found that the soy proteins were dissociated into subunits by HPP, some of which associated to aggregate and became insoluble; the denaturation of 7S and 11S were occurred at 300 MPa and 400 MPa, respectively, and induced tofu gels was formed with gel strength and a cross-linked network microstructure. Molina, Defaye and Ledward (2002) found that the HPP induced gels were formed in the range from 300 MPa to 700 MPa; compared to the thermal gels, the adhesiveness and hardness of HPP induced gels were significantly lower; the water holding capacity was improved by HPP in the gels of 7S glycinin. Tang and Ma (2009a) showed that the insoluble aggregate of soy protein isolate was formed at a lower pressure level (200 MPa), the insoluble aggregate was transformed into soluble aggregate at a higher pressure level (600 MPa), much more homogenous soluble aggregate was generated at 400 MPa or 600 MPa had much less mean molecular weight than that at 200 MPa, and the changes of secondary and tertiary structures were induced by HPP, that is the direct evidence or explanation for HPP induced modification of soy protein isolate. Kweon, Slade and Levine (2017) found that only a small effect on denaturation of the 7S soy globulin in 50% (w/w) soy flour-water paste was observed at 200 MPa (20 min, 25 °C), a significant effect on denaturation of both the 7S and 11S soy globulins was showed at 600 MPa. The other, a less-pronounced effect on denaturation of the 11S globulin was observed at 60 °C treated by different pressures, but a similar extent of denaturation of the 11S treated by 600 MPa at 25 °C and 90 °C was observed. The result showed that 7S is sensitive to heat and pressures combined, because it has a low denaturation temperature (68 °C); 11S is not sensitive to thermal, and sensitive to pressures, due to it has high denaturation temperature (96 °C). Thus, the application of thermal plus HPP could be used to produce enhanced food quality. In addition, the NaCl, sucrose, betaine, and lactobionic acid had a protective effect on protein denaturation during the HPP at 25 °C.

HPP and other materials combination also affected the processing properties of soy protein. Liu et al. (2020) investigated the effect of HPP (0.1–300 MPa) on soy protein isolate incubated with flaxseed gum at 60 °C for 3 d, the results shown that the solubility of soy protein isolate upon glycation with flaxseed gum was improved, the maximum value reached 86.84% when treated at pH 8.0 and 200 MPa, accompanied by producing the differences between the secondary structure of the glycated proteins and that of at 200 MPa, such as the α-helix, random coil contents and vibrations of the amide II band; at 100 MPa, the Maillard reactions were significantly promoted, to the contrary, the reactions were significantly suppressed at 200 MPa and over. Overall, proper pressure levels can improve the processing properties of soy protein. Chen et al. (2019) showed that HPP is a useful tool for improving the function of tea polyphenols and soybean proteins. The secondary structure of soy proteins was significantly modified at 400 MPa, such as increased the β-sheet content and decreased the α-helix content, but the α-helix structure was protected when the 0.1% (w/v) tea ployphenol was added. The other, the HPP and tea polyphenols combined could increase the solubility, emulsifying activity and micro-texture, the reason is that the Pi-Pi interaction was formed in the binding of phenolic compounds to 7S or 11S globular protein. Wang et al. (2011) found that the solubility of ethanol (EtOH)-denatured soy proteins at neutral and alkaline pH as well as low ionic strength was significantly improved treated above 200 MPa, the enthalpy value was increased and the ordered supramolecular structure with stronger intramolecular hydrogen bond was formed. Meanwhile, the Tyr and Phe residues were exposed, which caused an increase in surface hydrophobicity of 7S glycinin treated by HPP (200-400 MPa), but the surface hydrophobicity was decreased at 500 MPa. In contrast, the progressive unfolding of denatured glycinin was induced with increasing pressure, due to the Tyr and Phe residues were moved to the molecular surface of protein. Tang & Ma (2009b) reported that the secondary structure of native soy protein isolate is estimated to be composed of 15%-16% alpha-helix, 39%-44% extended strands, 17.5% random coils, and 21%-27% turns. At 200-400 MPa, the intensity and a "red-shift" of these bands were increased; at 600 MPa, the band intensity of the amide I' region was further increased, so that, the intensity and absolute area of amide II bands were gradual increases treated by HPP.

1.2.4 The effects of soy 7S and 11S by HPP

Soy 7S and 11S are determined by the emulsion capacity, foaming capacity, gel properties of soy protein. The changes in soy 7S and 11S were induced by the HPP (Molina, Papadopoulou & Ledward, 2001; Zhang, Li, Tatsumi & Kotwal 2003; Puppo, Speroni, Chapleau, de Lamballerie, Anon & Anton, 2005; Suzuki & Tada, 2011). Molina, Papadopoulou and Ledward (2001) showed that the highest emulsifying activity index and surface hydrophobicity of 7S globulin were treated at 400 MPa, the highest emulsifying activity index and surface hydrophobicity of 11S globulin were treated at 200 MPa, implying that the 7S globulin was dissociated into partially or totally denatured monomers at 400 MPa, which enhanced the surface activity; meanwhile, the pressure at 400 MPa induced the unfolding of the polypeptides of the 11S within the hexamer led to aggregation, which lowered the surface hydrophobicity.

The glycinin of soy was dissociated into subunits and the conformation of these subunits had been changed after HPP. At 300 MPa and over, the ultraviolet absorbance of hydrophobic regions, sulphydryl groups, and amino acid residues were changed significantly; at 400 MPa for 10 min, the denatured completely of glycinin was observed by DSC analysis; at 500 MPa for 10 min, the α-helix and β-sheet structures were destroyed and converted to random coil, thus, the pressure level was the influence of the conformational of soy glycinin (Zhang, Li, Tatsumi and Kotwal, 2003). Puppo et al. (2005) reported that HPP (200, 400 and 600 MPa for 10 min at 10 °C) induced more ability for proteins, and particularly β-7S and A-11S polypeptides, to be adsorbed at the oil–water interface. Suzuki and Tada (2011) found that the firmer mixture gel was formed after HPP, the gel strength and work done values were increased with the increase of pressure levels from 100 MPa to 400 MPa; over 400 MPa, the gel formation dropped dramatically; those indicated that the SH groups play a key factor in the gel of actomyosin and soy 11S under HPP. 7S and 11S globulin have different emulsion properties treated by HPP. Puppoa et al. (2011) found that 7S and 11S globulin emulsions (7%, w/v) behaved differently under the temperature (20–60 °C) and HPP (0.1–600 MPa) combined treatments, 7S globulin was responsible for the global properties of soy emulsions, whereas 11S globulin exerted a negligible effect; the 7S emulsions was increased the flocculation and gelation, which caused by aggregation between adsorbed and aqueous 7S proteins. The calcium and HPP combined was affected the thermal properties of soy protein isolate, a β-conglycinin-enriched fraction, a glycinin-enriched fraction, and whey protein concentrate. The T_d of glycinin was increased for every assayed calcium concentration, HPP promoted denaturation of β-conglycinin and glycinin, and calcium protected both proteins in β-conglycinin-enriched fraction and glycinin-enriched fraction at 200 MPa, protected glycinin in soy protein isolate and β-conglycinin-enriched fraction at 400 and 600 MPa (Speroni, Anon & Lamballerie, 2010 and 2014). Guan et al. (2018) reported that the hydrolytic efficiency of Corolase PP was increased and the surface hydrophobicity of the hydrolysates were decreased treated by HPP (80-300 MPa), the higher bioactivities of hydrolysates were observed under 200 MPa for 4 h, the small peptides (< 3 kDa) and the amino acid sequences of these peptides with different inhibitory abilities were increased, thus, HPP and Corolase PP combined could be used as a potential technology to produce bioactive peptides from soy protein isolate.

1.2.5 The effects of allergenicity from soy protein by HPP

HPP could reduce the allergenicity of soy protein isolate for infant formula. Recently, soy-based infant formula, as a replacement of milk for the lactose intolerant and cows' milk allergic infants, is being consumed more commonly, accounting for increased uptake all over the world (Fomon & Ziegler, 1979; Bhatia et al., 2008; Klemola et al., 2002). However, soy protein isolate contains some antigenic components, such as glycinin, α-conglycinin, β-conglycinin and γ-conglycinin. Some studies have reported the use of HPP to reduce allergenicity of soy seeds, whey protein, condensed soy glycinin and soy protein isolate (Peñas, Préstamo, Polo & Gomez, 2006; Peñas, Gomez, Frias, Baeza, & Vidal-Valverde, 2011; Savadkoohi, Bannikova, Mantri & Kasapis, 2016; Li, Zhu, Zhou & Peng, 2012; Li, Jia, Peng, Zhu, Zhou & Guo, 2018). The soy whey protein, as a by-product from the manufacture of tofu, has the antibodies against Gly m 1, which is an important allergen of soybean that causes allergy by inhalation, the immunoreactivity was decreased under 100–300 MPa for 15 min (Peñas, Préstamo, Polo & Gomez, 2006). Savadkoohi, Bannikova, Mantri and Kasapis (2016) showed that the soy glycinin with twelve disulphide linkages displays extensive unfolding at low to intermediate solid levels (30–

60%, w/w), but it largely maintains native conformation at 70% and 80% (w/w) solids showing about 20% denaturation under 600 MPa for 15 min at ambient temperature, as compared to the thermal transition of native counterparts. Torrezan, Frazier & Cristianini (2010) studied the effects of HPP (200-700 MPa) on antinutritional factors phytate and trypsin inhibitor content in 5% soy protein isolate solution, and who found that the phytate was efficient to eliminate treated by HPP, but the trypsin inhibitor content was not changed. Li, Zhu, Zhou and Peng (2012) found that the processing pressure and duration time could significantly influence the allergenicity reducing efficiency. Such as, the allergenicity of soy protein isolate decreased 48.6% compared to the native under 300 MPa and 15 min, the reason is that the free SH content and hydrophobicity of soy protein isolate were significantly increased under 200–300 MPa for 5–15 min; the two interactions were progressively decreased treat by the levels above 300 MPa for 15 min; the secondary structure of soy allergens interfered and the allergenicity by modifying conformation of allergenic epitopes were decreased after HPP. Li et al. (2018) utilized the method of proteomics to confirm allergen subunit differences of soy protein isolate between control and HPP, and found that the allergenicity was decreased by 45.5% at 300 MPa for 15 min, and altered the allergenicity of α and α′ subunits of 7S globulin and A1 and A1a subunits of 11S globulin, so that, the use of HPP could improve the safety of soy protein in infant formula.

It is well established that HPP improved the properties of soy protein, 7S and 11S glycinins. The proper pressure treatment of soy protein, 7S and 11S glycinins increased the water holding capacity, gel and emulsion properties through affected the non-covalent bond, covalent bond and protein conformation, and also reduced the allergenicity of soy proteins in infant formula. In spite of great efforts, the mechanism of HPP on soy protein, 7S and 11S glycinins has not yet been obtained, which leads to get a clear understanding of their behaviour is difficult. Therefore, the aim of this section is to investigate the application of HPP and soy protein isolate to modify the properties of meat products, increase the water holding capacity and texture of cooked gel meat products, and then the used of HPP and soy protein isolate combinations to emulsion meat products could improve the quality and lower the salt content in the meat industry.

Conclusions in section 1

1. We can confirm that HPP improves the properties of muscle, comminuted meat and myofibrillar proteins, and found that the use of moderate pressure treatment of pre-rigor meat seems to have potential since the meat will be tender and look normal color. Reasonable high pressure processing could enhance the water holding capacity and texture of comminuted meat, but the products lacked the cooked appearance and potential for accelerated loss of flavour.

2. It is proven that the use of high pressure processing could improve the quality of reduced-salt meat products through affected the non-covalent bond, covalent bond and protein conformation of myofibrillar proteins, the water holding capacity and texture of myofibrillar proteins will be increased produced by moderate pressure treatment.

3. We have established that HPP improved the properties of soy protein isolate. The proper pressure treatment of soy protein was increased the water holding capacity, gel and emulsion properties through affecting the non-covalent bond, covalent bond and protein conformation, and also reduced the allergenicity of soy proteins in infant formula.

4. The main processing and materials of reduced-salt meat products are determined, in accordance with the references. Thus, HPP and soy protein isolate combined can be used to create reduced-salt meat products.

5. The review provides a theoretical basis for our experimental design.

SECTION 2.

ORGANIZATION, SUBJECTS, MATERIALS AND METHODS RESEARCH

2.1 Objects of research

2.1.1 Raw materials and ingredients

The *longissimus dorsi* of chilled pork [*Duroc* × *(Landrace* × *Yorkshire)*] (Moisture, 71.35±0.52%; protein, 22.57±0.37%; fat; 2.83±0.26%; pH, 5.63±0.02) were derived from the landrace (100±5 kg) which were slaughtered at the age of about 6 months provided by the Gaojin Group (China), and the temperature after slaughter 24 h was 2~4 °C. After removing of the visible connective tissue and fat, the pork meat was minced using a meat chopper with a 6 mm holes plate (MGB-120, Shandong Jiaxin Food Machinery Co., Ltd., China). The ground meat (400 g each) was packaged in double plastic (nylon/PE) bags and stored at -20 °C until use within 2 weeks. Pork back-fat (90.21±0.56% fat) was purchased from a local meat market (Xinxiang, China), and also was minced using a meat chopper with a 6 mm holes plate. Soy protein isolate (91.32±0.83% protein) was provided by Shandong Soy Foods co., Ltd (China). Pork myofibrillar protein was homemade from *longissimus dorsi* of chilled pork.

Tris, EDTA, KCl, $MgCl_2$, NaCl, K_2HPO_4, KH_2PO_4, EGTA, NaN_3, Triton, urea, and glycine were analytical grades.

All the raw materials and ingredients meet the requirements of the current regulatory documentation.

2.2 Research methods

2.2.1 Prepared meat batters

All the raw pork batters were prepared with 400 g pork meat, 80 g pork back-fat, 73.5 g ice water, 1% NaCl. In addition, and then added 0%, 2%, 4% soy protein isolate, respectively. The pork batters were produced by a bowl chopper (Stephan UMC-5C, Germany). The ground meat was thawed overnight at 4 °C prior to use. Briefly, the thawed meat (400 g each), NaCl and 36.75 g ice water was chopped (1500 rpm) for 30 s; and then added 80 g pork back-fat chopped (1500 rpm) for 30 s; prior to finishing with a high speed (3000 rpm) emulsification for 60 s, and the 36.75 g ice water was continue to add for keeping the final temperature less than 10 °C. Immediately after chopping, the batter was stuffed by a vacuum stuffer (VF608, Germany), in 24 mm diameter edible collagen sausage casings (Shenguan Holdings (Group) Limited, China). Pork batters were hand linked at 18 cm intervals, and weighed. Finally, the batters were vacuum packed for subsequent pressure processing.

2.2.2 Extraction of myofibrillar protein and preparation of mixed protein solutions

The myofibrillar protein was extracted from 2 kg of ground pork meat. First, the ground meat was thawed overnight at 4 °C prior to extracting. Next, the ground meat was homogenized in four volumes of a buffer (100 mmol·L^{-1} Tris, 10 mmol·L^{-1} EDTA, pH 8.3) in a homogenizer (T25, IKA, Germany). The homogenates were centrifuged (4 °C) at 1000×g for 20 min (Sorvall LYNX4000, Thermo Fisher Scientific, Germany). Removed the supernatant and the sediments were resuspended in four volumes of a buffer (100 mmol·L^{-1} KCl, 20 mmol·L^{-1} K_2HPO_4/KH_2PO_4, 2 mmol·L^{-1} $MgCl_2$, 1 mmol·L^{-1} EGTA, 1 mmol·L^{-1} NaN_3, pH 7.0) and centrifuged at 1000×g for 10 min under the same conditions above for another twice. After that, the sediments were resuspended in four volumes of another buffer (100 mmol·L^{-1} KCl, 20 mmol·L^{-1} K_2HPO_4/KH_2PO_4, 2 mmol·L^{-1} $MgCl_2$, 1 mmol·L^{-1} EGTA, 1 mmol·L^{-1} NaN_3, 1 % Triton X-100, pH 7.0), and then centrifuged (1500×g for 10 min) at 4 °C. Removed the supernatant, sediments were resuspended in four volumes of 0.1 mol·L^{-1} KCl solution and centrifuged at 1500×g for 10 min (4 °C). Next, sediments were resuspended in four volumes of 0.1 mol·L^{-1} NaCl solution and centrifuged at 1500×g for 10 min (4 °C). Finally, the purified myofibrillar protein sediment was obtained, stored at 4°C and used within 24h. The protein content was measured by the Biuret method using bovine serum albumin (BSA) as the standard.

The myofibrillar protein was diluted to 60 mg/mL [dissolved in 50 mmol/L K_2HPO_4/KH_2PO_4 with 1% NaCl (Weight of NaCl/Weight of myofibrillar protein solution), pH 6.0], respectively. Then, the 0% (C1), 2% (C2), and 4% (C3) soy protein isolate (Weight of soy protein isolate/Weight of myofibrillar protein solution) was added to the 100 mL myofibrillar protein solutions, which were loaded into a 200 mL beaker, respectively. The protein solutions were mixed uniformly using a homogenizer (T25, IKA, Germany) in an ice bath, according to the following procedure: 3000 rpm, 20 s; and 5000 rpm, 50 s. The solutions were then centrifuged at 500×g (4 °C, 3 min) to remove any air bubbles, vacuum conditioned in a bag, and stored at 2±2 °C for at least 12h to allow maximum protein dissolution.

2.2.3 High pressure treatment

The batters or myofibrillar proteins were treated by different pressures using a high pressure vessel (S-FL-850-9-W/FPG5620YHL, Stansted Fluid Power Ltd., Stansted, UK), the temperature was controlled through a thermo-stating circulator water bath. The high pressure procedure was as follows: pressure, 0.1, 100, 200, 300, and 400 MPa; time, 10 min; temperature, 10±2 °C. The compression rate was approximately 3 MPa/s, and the decompression step was reached immediately (< 3 s). Following, all batters (apart from those used for rheology measurement) were heated at 80 °C for 30 min until the internal temperature 72 °C. Immediately, the cooked batters were cooled by running water and stored at 4 °C.

2.2.4 Determination of texture

The texture profile analysis of cooked pork batters (the cylindrical-shaped with a diameter of 20 mm and a height of 20 mm) or cooked myofibrillar protein solution was carried out using a texture analyzer (TA-XT plus Texture analyzer, Stable Micro Systems, UK) with an aluminum cylindrical probe P/36R, and provided with the instrument (Fig. 2.1). Parameters as follow: pre-test speed 2 mm/s, test speed 2 mm/s, post-test speed 2 mm/s, compression ratio 40 %, trigger force 5 g, and 5 s was allowed between the two compression cycles. The indicators of hardness, springiness, cohesiveness and chewiness (hardness × cohesiveness × springiness) were determined. Each measurement was replicated 5 times.

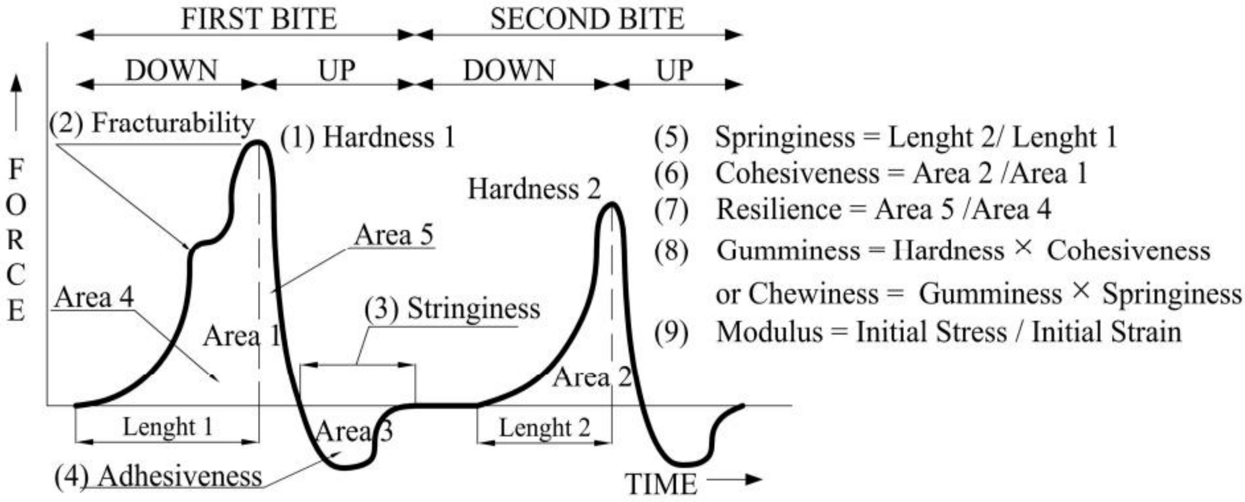

Fig. 2.1 The parameter definition interpretation of qualitative curve analytical method

Hardness. It is the biggest peak of compression for the first time.

Springiness. The quotient or volume ratio of the compressed deformed sample to the preformed condition after removing the deforming force. Elasticity is expressed by the ratio (Length2/ Length1) of the specimen recovery height (Length 2) measured in the second compression to the first compression deformation (Length 1).

Cohesiveness. The relative resistance of the test sample to the second compression after the first compression deformation is shown in the curve as the ratio of positive work (Area 2/Area 1) of the two compressions. This value represents the total work required to overcome the attraction between the two surfaces when the probe comes into contact with the sample.

Chewiness. It is only used to describe the test sample in a solid state, indicating the energy required to chew the solid sample into a stable state when swallowing. The numerical value is expressed by the product of the stickiness and elasticity (hardness × cohesive elasticity).

2.2.5 Low field NMR measurements

Low field NMR relaxation measurements were carried out according to the method of Kang et al. (2017). About 2 g of the cooked pork batter or myofibrillar protein was placed in a 15 mm glass tube and inserted in the NMR probe of a Niumag Pulsed NMR analyzer (PQ001, Niumag Electric Corporation, Shanghai, China). Spin-spin relaxation time (T2) was measured making a τ-value of 350 μs by the Carr–Purcell–Meiboom–Gill sequence at a resonance frequency of 22.6 MHz, 32 °C. Post processing of T2 data distributed exponential fitting of Carr-Purcell-Meiboom-Gill decay curves was performed by Multi-Exp Inv Analysis software (Niumag Electric Corp., Shanghai, China). Each measurement was replicated 4 times.

2.2.6 Determination of colour

The colour (L^*, a^* and b^* values) of cooked batter or myofibrillar protein core was measured by a colourimeter (CR-400, Minolta, Japan), the calibrated white plate is L^*=96.86, a^*=-0.15, b^*=1.87.

2.2.7 Determination of pH

About 10g cooked batter or myofibrillar protein and 40mL distilled water were homogenized at 15,000 rpm, 10 s. Immediately, the pH of solution was measured by a pH meter (Hanna, Italy). All analyses were carried out in triplicate.

2.2.8 Determination of emulsion stability

Emulsion stability of pork batters was measured as follows: approximately 25 g raw batter was put in a 50 mL centrifuge tube and centrifuged at 500×g at 4 °C for 15 min (Model 225, Fischer Scientific, Pittsburgh, Pa., U.S.A.) to eliminate any air bubbles. Each sample was cooked in an 80 °C water bath for 20 min, then removed from the water bath, uncapped and the left inverted for 50 min on paper tissues to release any exudate at 20 °C. The total fluid released (TR) was expressed as % of the initial sample weight; the smaller the TR, the better the emulsion stability. The water released component (WR, % of the initial sample weight) was determined from the dry matter content of the TR after heating at 105 °C for 16 h. The fat released component (FR, % of the initial sample weight) ignored any minor protein or salt components and was taken as the difference between TR and WR.

2.2.9 Determination of cooking yield

The cooking yield of pork batters or myofibrillar protein was calculated according to the following formula:

Cooking yield (%) = cooked meat batter/raw meat batter × 100%   (2.1)

Each measurement was replicated 5 times.

2.2.10 Determination of rheology

Dynamic rheological studies were performed on a HAAKE MARS dynamic rheometer (Thermo Scientific, American). A P35TiL parallel steel plate geometry with a 0.5 mm gap was used. The raw batter or myofibrillar protein was placed between the flat parallel plates with its perimeter coated with a thin layer of silicone oil to prevent dehydration. Samples were heated at a rate 2 °C/min from 20 °C to 80 °C. During this heating process, the sample was continuously sheared in an oscillatory mode at a fixed frequency of 0.1 Hz. Changes in the storage modulus (G' i. e. the rigidity due to the elastic response of the material) were measured during the process with increasing temperatures (either elastic or storage modulus (G') was recorded). Each sample was measured in triplicate.

2.2.11 Raman spectroscopic analysis

Raman experiments of the cooked batters were measured by a procedure of Zhu et al. (2018). The cooked batters were spread on a glass slide before measurement. The spectra were obtained in the range of 400 cm^{-1} to 4000 cm^{-1}. Each spectrum of cooked batters was determined under the following conditions: three scans, 30 s exposure time, 2 cm^{-1} resolution, sampling speed 120 cm^{-1}/min, and data collection every 1 cm^{-1}. Spectra were smoothed, baselines corrected and normalized against the phenylalanine band at 1003 cm^{-1}. The result showed the changes in secondary structures of pork proteins as percentages of α-helix, β-sheet, β-turn, and random coil.

2.2.12 Total and reactive sulfhydryl groups

The total and reactive sulfhydryl groups were measured following the methods of Ellman (1959) with some modifications. Briefly, the myofibrillar protein solution with varying soy protein isolate addition was diluted into 5 mg/mL, and it was treated for 10 min under 200 MPa, at 10±2 °C. After that, 1.5 mL myofibrillar protein solution (5 mg/mL) was suspended in 10.0 mL of Tris-glycine buffer (0.086 mol/L Tris, 0.09 mol/L glycine, 4 mmol/L EDTA, 8 mol/L urea, pH 8.0). A 50 μL of Ellman reagent (4 mg DTNB was dissolved in 1 mL Tris-glycine buffer) was added to all the above treatments. The water bath was applied for 1 h at 25±1 °C after the vortex oscillation. After centrifugation at 12000×g for 10 min, the supernatant was obtained, and the absorbance was measured at 412 nm. As for the calculation, an extinction coefficient of 13600 M^{-1} cm^{-1} was applied.

2.2.13 Surface hydrophobicity

The surface hydrophobicity of myofibrillar protein solutions with various amounts of soy protein isolate was measured following the methods of Yong sawatdigul and Park (2003). The specific operation was as follows: the myofibrillar protein solution was adjusted to 2 mg/mL with 50 mmoL/L phosphate buffer (pH, 6.0). The sample (1 mL) was vortexed for 10 min after mixing with 0.2 mL BPB (1 mg/mL). The supernatant was taken after centrifugation at 2000×g for 15 min, and the bound of BPB was monitored using a UV-scanning spectrophotometer at 595 nm after a dilution of 10 times. The BPB bound (H_0) of the protein surface was calculated according to the following formula:

$$\text{BPB bound } (\mu g) = 200\ \mu g \times (OD_{control} - OD_{sample}) / OD_{control} \quad (2.2)$$

2.2.14 Cool storage

All the cooled cooked batters were stored at 2±2 °C for 60 days. Analyses were performed at the 1st, 30th and 60th days. Therein, the samples of C were cold stored at the 1st, 30th and 60th days named as C1st, C30th and C60th; the samples of T were cold stored at the 1st, 30th and 60th days named as T1st, T30th and T60th, respectively.

2.2.15 Determination of storage loss

Storage loss of cooked batters was measured at 1st, 30th and 60th days, respectively. Sample with casing was weighed (weight sample). The surface water of the product was absorbed using filter paper and reweighed (weight product). Storage loss was calculated as a percentage of the original weight. The calculation formula is shown as follows:

$$\text{Storage loss } (\%) = (\text{weight sample} - \text{weight product})/\text{weight sample} \times 100 \quad (2.3)$$

2.2.16 Determination of total plate count

A 10 g sample of pork batter was ground in a sterile pestle and mortar with 90 mL sterile 0.1% peptone water. Appropriate dilutions of samples were prepared in sterile 0.1% peptone water and plated, in duplicate, on the growth media by using the pour plate method. Plate count agar was used for total plate count. The plates were incubated at 35±2 °C for 24 h, colonies were counted and expressed as log10 CFU.g^{-1} sample.

2.2.17 Determination of TBARS

According to the method of Ulu (2004), the changes in TBARS of cooked batter were determined. Approximately 10 g pork batter was homogenized and transferred to a Kjeldahl flask followed by the addition of 97.5 mL of distilled water and 2.5 mL of 6 N HCl. The mixture was heated with steam distillation until 50 mL of distillate was collected. 5mL of distillate was added to 5mL of thiobarbutiric reactive reagent containing 0.02 mol/L TBA in 90% glacial acetic acid and incubated in boiling water for 35 min. After cooling with tap water, the absorbance of the pink solution was read at 538 nm. The constant 7.8 was used to calculate the distillation TBARS number.

2.3 Planning an experiment and conducting a Research

To ensure a clear and consistent implementation of theoretical and research works, a detailed research plan was developed. (Fig. 2.2). It provided for a theoretical justification for the development of new technology to produce reduced-salt pork meat batter using HPP and soy protein isolate combinations. and to ensure a clear and consistent implementation of theoretical and research works, a detailed research plan was developed (Fig. 2.2). It provided for a theoretical justification for the development of a new reduced-salt pork meat batter using the technology of HPP and soy protein isolate combinations, determination of the optimal pressure levels and soy protein isolate additional, development of technological schemes of reduced-salt pork meat batter, experimental research on the study of their quality and shelf-life, in the production of packing stations.

Theoretical researchers were carried out in the following areas. Review of domestic and foreign literature on the problems of reducing salt in meat products, using the HPP and soy protein isolate to produce meat products, and ways to solve it; analysis of properties and existing technologies of reduced-salt meat products, and modified the muscle protein and soy protein isolate using HPP; analysis of prospects for the use of the technology of HPP and soy protein isolate combinations in reduced-salt meat products.

On the basis of the theoretical research was formulated the main purpose of the study and individual tasks of its achievement. In the first stage, determined the water holding capacity, techno-functional properties and protein conformation of reduced-sodium pork meat batters with different soy protein isolate which were produced by HPP, and thereby establishing a method to obtain pork meat batter with desirable quality.

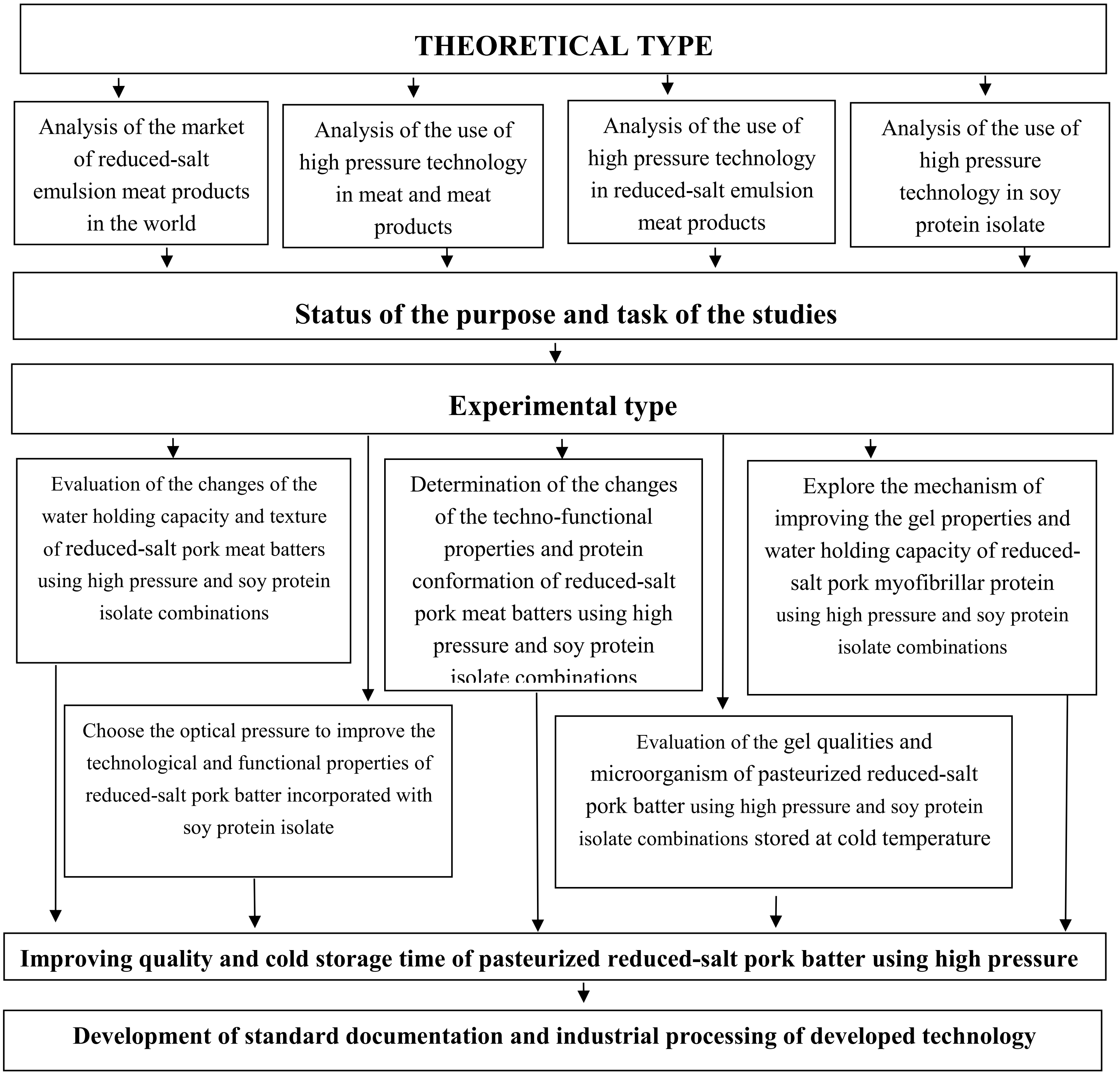

Fig. 2.2. The technology road map about planning an experiment

In the second stage, complete the experiment of "Effects of soy protein isolate on gel properties and water holding capacity of reduced-salt pork myofibrillar protein under high pressure processing", and understand the mechanism of how to improve the techno-functional properties of pork myofibrillar protein using the technology of HPP and soy protein isolate combinations, especially the changes of gel properties, water holding capacity and protein conformation.

In the third stage, complete the experiment of “Techno-functional properties, water distribution and mobility of reduced-salt pork batters with soy protein isolate as affected by pressures”, to evaluate the changes of techno-functional properties and water holding capacity of reduced-salt pork batter under different pressures.

In the fourth stage, complete the experiment of “Effects of soy protein isolate on gel properties and water holding capacity of reduced-salt pork myofibrillar protein under high pressure processing”, the directions were determined and recommendations for the use of the technology of HPP and soy protein isolate combinations for the production of reduced-salt pork batter were developed. The fifth stage was the implementation of research results in practice.

The experimental part of the dissertation was carried out in laboratories of Food Technologies Faculty of Sumy National Agrarian University (Sumy, Ukraine), Henan Institute of Science and Technology “National Pork Research and Development Technology Center ” (Xinxiang, PR China).

Industrial approbation of the results of the study was carried out in production conditions on the basis of the factory in Nanjing Huang Professor Science and Technology Food Co. LTD, (China), Henan Zhongpin Food Industry Co. LTD, (China), and Hua County Ji Xianda Food Co. LTD, (China).

2.4 Statistical processing of research results

The experiment was repeated four times on different occasions (n=4), using different samples (pork batters or myofibrillar protein solutions with 0%, 2% and 4% soy protein isolate). In each replication, three different protein solution formulations were prepared. The data were analyzed through the general linear model (GLM) procedure using the statistical software package SPSS v.18.0, considering the treatments (different soy protein isolate content) as a fixed effect and the replicates as a random effect. Significant differences between means were identified by the LSD procedure. The results in the tables and figures are expressed as mean values and standard errors (mean ± SE). The difference between means was considered significant at $P < 0.05$.

Conclusions in section 2

1. Methodological approaches adopted in the dissertation work that include theoretical research, raw materials and ingredients, experiment methods, practical testing, technology promotion, subordinate various methods of research for the only goal decision set in the dissertation work scientific problem.

2. The object of research of dissertation work is determined. The use of high pressure processing and soy protein isolate combined improve the techno-functional properties of reduced-salt pork batter, to produce the reduced-salt pork batter with good quality, such as reduced-salt sausages and meatballs.

SECTION 3.

EFFECT OF SOY PROTEIN ISOLATE ON THE WATER HOLDING CAPACITY, TECHNO-FUNCTIONAL PROPERTIES AND PROTEIN CONFORMATION OF LOW-SODIUM PORK MEAT BATTERS TREATED BY HIGH PRESSURE

Pressure, volume, and protein material due to structural differences appear to different degrees of compression deformation, when the deformation degree is large enough. It may affect the combination between protein molecules form, causes the destruction and restructuring of key, which also affects the functional characteristics of protein. Moreover, protein molecule conformation may bring better functional characteristics due to the sudden release of pressure after pressure is withdrawn. An increase in water holding capacity due to the interaction of HPP and salt in pork meat batter, may be because increasing sodium chloride causes increasing denaturation of muscle proteins in HPP treated meat batters and favors the solubilization of proteins and the formation of a gel network that retains water and fat. Soy protein isolated as the ingredients is widely used in low temperature emulsified meat products, because it has the moisture adsorption and machining process of moisture keeping ability. How to add soy protein isolate without reducing the quality of emulsified meat products is a difficult problem that needs to be solved urgently. Therefore, the aim of this section was to determine the water holding capacity, techno-functional properties and protein conformation of reduced-sodium pork meat batters with different soy protein isolate which were produced by HPP, and thereby to establish a method obtain pork meat batter with desirable quality.

3.1 Cooking yield

The effect of HPP with various amounts of soy protein isolate on the cooking yield of pork batters is shown in Fig. 3.1. A higher cooking yield of pork batters reflects a better water holding capacity. We can observe that all the cooking yield of pork batters with various amounts of soy protein isolate were increased significantly ($P < 0.05$) compared with the C1, but the cooking yields of C1 and C2 were not significantly ($P < 0.05$) different.

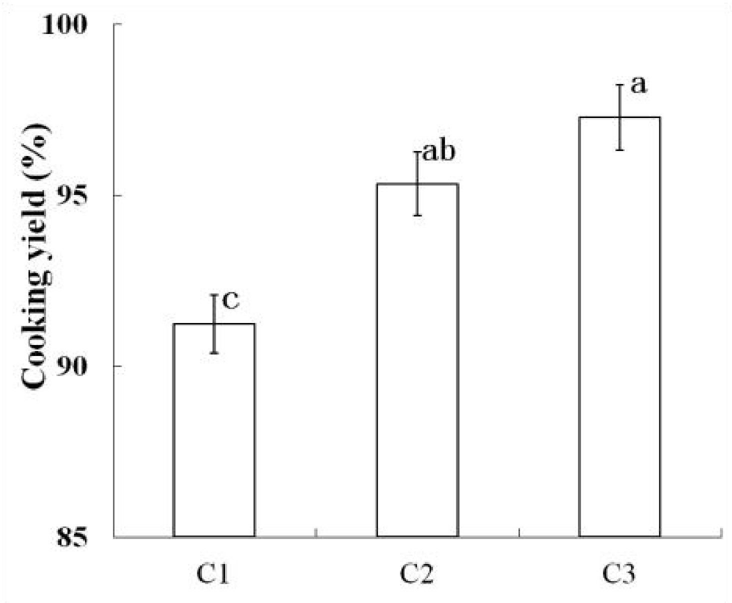

Fig. 3.1 Effect on cooking yield (%) of pork meat batters by high pressure processing with different soy protein isolate.

C1: 0% soy protein isolate; C2: 2% soy protein isolate; C3: 4% soy protein isolate.

Each value represents the mean ± SD, n = 4.

[a-c] Different parameter superscripts in the figure indicate significant differences ($P < 0.05$).

We assumed the reason is that adding the 2% soy protein isolate could hold the water of pork batters very well, so increasing the soy protein isolate addition could not improve the cooking yield. The other, the emulsifying activity of 11S globulins were significantly improved at 200 MPa, which enhanced the water holding capacity of soy protein isolate. The use of the soy protein isolated, wheat flour, and κ-carrageenan as a binder, showed that the addition of binders improved water-binding properties of pressure or non-pressure-induced restructured pork. The use of the dried egg white as a fat replacement to obtain a low-fat chicken gel by means of HPP, the water binding properties and hardness were improved, suggesting their participation in the network structure coupled to the myofibrillar proteins, and noted that the modifying certain functional characteristics of chicken meat gels with low fat content by means of HPP and the addition of dried egg white. Thus, we established the addition of soy protein isolate could improve the cooking yield of pork batters.

3.2 Texture properties

The texture of cooked pork batters was affected significant ($P < 0.05$) by HPP and soy protein isolate combinations (Table 3.1).

Table 3.1 - Texture of cooked pork batters by high pressure processing with different soy protein isolate

Sample	Hardness (N)	Springiness	Cohesiveness	Chewiness (N mm)
C1	47.32±1.12^{c}	0.837±0.008^{b}	0.641±0.005^{c}	27.05±0.85^{c}
C2	53.21±0.98^{a}	0.863±0.009^{a}	0.687±0.007^{a}	35.68±0.89^{a}
C3	50.42±1.05^{b}	0.835±0.007^{b}	0.655±0.008^{b}	29.67±0.96^{b}

C1: 0% soy protein isolate; C2: 2% soy protein isolate; C3: 4% soy protein isolate.

Each value represents the mean ± SD, n = 4.

$^{a-c}$ Different parameter superscripts in the table indicate significant differences ($P < 0.05$).

We observed that all the hardness, springiness, cohesiveness and chewiness of pork batters with various amounts of soy protein isolate were increased significantly ($P < 0.05$) compared with the C1, except for the springiness of C3. Compared with the C3, the hardness, springiness, cohesiveness and chewiness of pork cooked batter with 2% soy protein isolate (C2) were significantly increased ($P < 0.05$). HPP processing induced texture modifications have been used to affect myofibrillar proteins and their gel-forming properties, raising the possibility of the development of processed comminuted meat products. Over 200 MPa treatment, the protein extractability was decreased significantly in meat batters, and caused protein denaturation and/or aggregation, which limited their functionalities. Although the soy protein isolate has good water- and fat- holding capacity, and excellent gelling and structuring behaviour, some papers have reported that excessively adding the soy protein isolate could lower the texture of meat batters. Therefore, we obtained that the pork cooked batter with 2% soy protein isolate had the best texture.

3.3 Low field NMR

The effects of relaxation time and peak ratio of cooked pork batters by HPP processing with different soy protein isolate were determined (Fig. 3.2 and Table 3.2). We can observe that there were three characteristic peaks in the cooked pork batters, which were named as T_{2b}, T_{21} and T_{22}, respectively. T_{2b} is assigned to water tightly associated with protein and macro-molecular constituents, the relaxation population centered at approximately 0-10 ms in the cooked pork batters. The relaxation population of T_{21} is centered at approximately 10-100 ms, which is a major component and considered to intra-myofibrillar water and water within the protein structure. T_{22} corresponds to extra-myofibrillar water and centered at approximately100-400 ms. Compared with the C1, the initial relaxation times of T_{2b}, T_{21} and T_{22} were quicker ($P < 0.05$) in the C2 and C3, the result indicated that the cooked pork batters made with various amounts of soy protein isolate were bound tightly, because the changes of fast relaxing protein and slowly relaxing water protons.

These also were in accordance with the changes of texture and cooking yield (Table 3.1 and Fig. 3.1). We assumed the reason is possible that the soy protein isolate had excellent gelling and structuring behavior, then a better gel structure of cooked pork batters by HPP was formed when added the soy protein isolate. The emulsifying activity of 11S globulins of soy protein isolate was significantly improved at 200 MPa, through the changes of protein solubility, surface hydrophobicity, free SH content and secondary structure. All the peak ratios of T_{2b} were no significant differences ($P > 0.05$), C2 and C3 had the smallest peak ratios of T_{22}, and had the largest peak ratio of T_{21}. Added the soy protein isolate and HPP processing combinations could increase the protein content, more meat proteins can become available for gel formation of the meat matrix. These caused the water tightly associated with protein and macromolecular constituents to decrease, and improve water holding capacity of cooked meat batters. Therefore, adding the soy protein isolate increased the water holding capacity, and improved the texture of cooked meat batters.

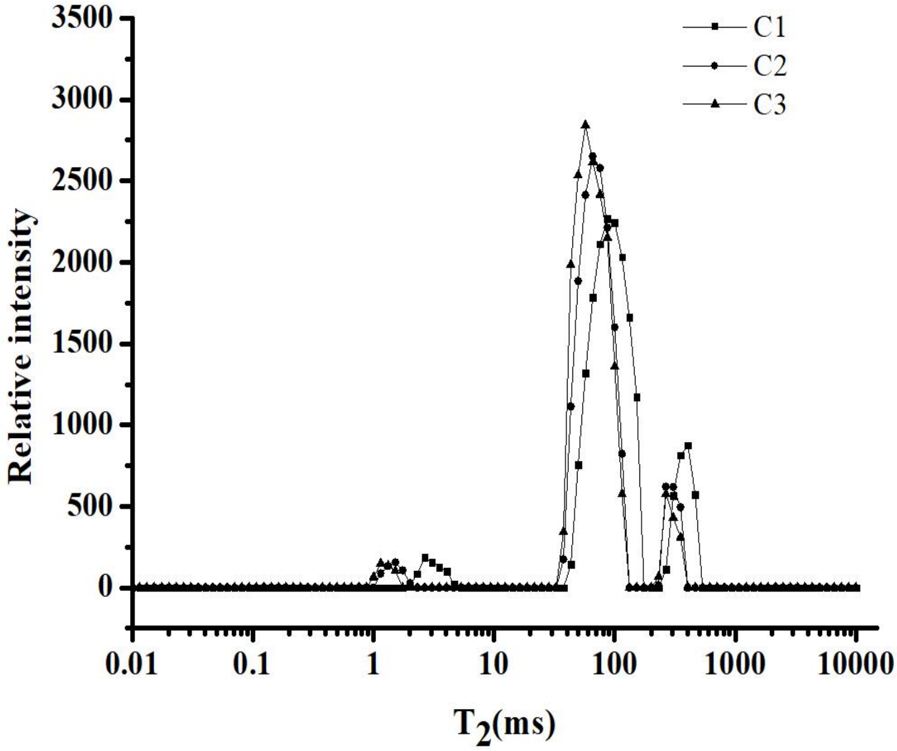

Fig. 3.2 Curves of relaxation time (T_2) in cooked pork batters by high pressure processing with different soy protein isolate.

C1: 0% soy protein isolate; C2: 2% soy protein isolate; C3: 4% soy protein isolate.

Each value represents the mean ± SD, n = 4.

Table 3.2 - Relaxation time (ms) and peak ratio (%) of cooked pork batters by high pressure processing with different soy protein isolate

Sample	Relaxation time (ms)			peak ratio (%)		
	T_{2b}	T_{21}	T_{22}	T_{2b}	T_{21}	T_{22}
C1	1.95±0.13^a	44.23±1.42^a	265.51±4.26^a	1.22±0.15^a	85.66±2.36^b	13.26±0.85^a
C2	1.12±0.15^b	37.25±1.59^b	232.87±4.68^b	0.96±0.12^a	91.87±2.45^a	8.31±0.80^b
C3	1.06±0.11^b	36.30±1.45^b	227.52±4.31^b	1.03±0.12^a	93.26±2.14^a	7.03±0.86^b

C1: 0% soy protein isolate; C2: 2% soy protein isolate; C3: 4% soy protein isolate.

Each value represents the mean ± SD, n = 4.

$^{a-b}$ Different parameter superscripts in the table indicate significant differences ($P < 0.05$).

3.4 Instrumental colour

Table 3.3 - Effect on colour of pork meat batters by high pressure processing with different soy protein isolate.

Sample	L^* value	a^* value	b^* value
C1	58.92 ± 1.45^{b}	6.27 ± 0.60^{a}	9.52 ± 0.45^{c}
C2	62.35 ± 1.68^{a}	4.65 ± 0.49^{b}	11.26 ± 0.57^{ab}
C3	64.21 ± 1.44^{a}	4.13 ± 0.53^{b}	12.55 ± 0.48^{a}

C1: 0% soy protein isolate; C2: 2% soy protein isolate; C3: 4% soy protein isolate.

Each value represents the mean ± SD, n = 4.

$^{a\text{-}c}$ Different parameter superscripts in the table indicate significant differences (P < 0.05).

The effect of HPP processing and added soy protein isolate on the colour of batters is shown in Table 3.3. The result showed that all the L^* and b^* values of pork batters with various amounts of soy protein isolate were significantly increased (P < 0.05) compared to the C1, the a^* values were decreased significantly (P < 0.05), but the L^*, a^* and b^* values of C2 and C3 were not significantly different (P > 0.05). HPP could induce the changes in functional properties of soy protein isolate, such as improved solubility, water holding capacity, emulsification activity index, and foam capacity. In this experiment, we found that the pork back-fat globules were covered by soy protein isolate after adding the soy protein isolate, and formed smaller back-fat globules which had more surface area and then reflect more light. The L^* and b^* values of frankfurters were increased, the a^* value was lowered when added pre-emulsified soy oil with soy protein isolate.

3.5 pH

The pH of cooked pork batters was affected significantly (P < 0.05) by HPP and soy protein isolate combinations (Fig. 3.3). We found that the pH was significantly increased (P < 0.05) when added soy protein isolate. The main reason was that the pH of soy protein isolate was 7.21±0.06, which could improve the pH of cooked pork batters. But there were no significant differences (P > 0.05) with soy protein isolate increased. The other reason was possible that more alkaline residues of pork batters were exposed which were treated by HPP and soy protein isolate combinations.

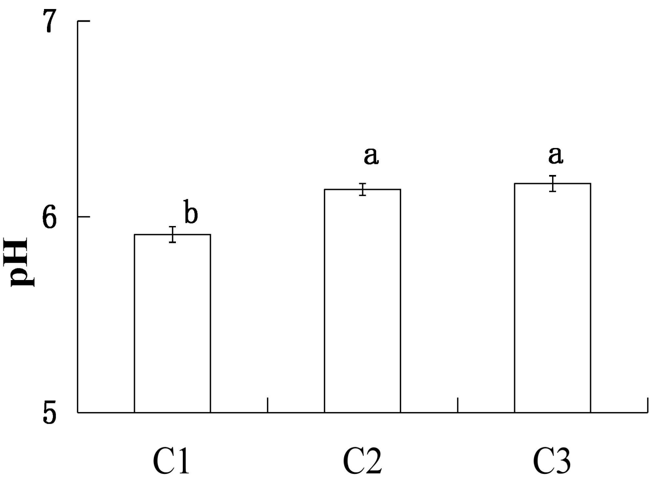

Fig. 3.3 Effect on pH of pork meat batters by high pressure processing with different soy protein isolate.

C1: 0% soy protein isolate; C2: 2% soy protein isolate; C3: 4% soy protein isolate.

Each value represents the mean ± SD, n = 4.

$^{a\text{-}b}$ Different parameter superscripts in the figure indicate significant differences ($P < 0.05$).

3.6 Emulsion stability

The effects of emulsion stability of pork batters by HPP with different soy protein isolate were determined (Table 3.4). The TR, WR and FR were significantly decreased ($P < 0.05$) with soy protein isolate increased. It is well known that as a binder or filling agent of commonly useful vegetable protein in the meat industry, soy protein isolate has good water and fat holding capacity, and excellent gelling and structuring behaviour. Meanwhile, the emulsifying activity of 11S globulins was significantly improved at 200 MPa, that enhanced the emulsified stability of soy protein isolate. Due to the ability of soy protein isolate to form gels with good water and oil holding capacity during heating, which improved the stability of pork meat batter and decreased the TR, WR and FR with the increase in content of soy protein isolate. The result was in agreement with the result of cooking yield and low field NMR, we exhibited that the pork batters with soy protein isolate had less water out the cooked pork batter and free water, these were in agreement with the emulsified stability of pork batters.

Table 3.4 - Effect on emulsion stability of pork meat batters by high pressure processing with different soy protein isolate.

Sample	TR (%)	WR (%)	FR (%)
C1	10.21±0.65[a]	8.85±0.46[a]	1.56±0.21[a]
C2	6.03±0.58[b]	5.32±0.41[b]	0.74±0.17[b]
C3	4.27±0.71[c]	3.84±0.52[c]	0.36±0.18[c]

TR: total fluid release; WR: water released component (% of the initial sample weight); FR: fat released component (% of the initial sample weight).

C1: 0% soy protein isolate; C2: 2% soy protein isolate; C3: 4% soy protein isolate.

Each value represents the mean ± SD, n = 4.

[a-c] Different parameter superscripts in the table indicate significant differences ($P < 0.05$).

3.7 Dynamic rheology

The G′ values of raw meat batters treated by HPP with different soy protein isolate is shown in Fig. 3.4. C1, C2 and C3 had similar heating curves and three phases evident from 20 °C to 80 °C. Because of the gelation was initiated following, increased from 42 °C to 51 °C (C1), 43 °C to 52 °C (C2), and 43 °C to 52 °C (C3), respectively. The main proteins in soy protein isolate are glycinin (11S) and β-conglycinin (7S), which usually are present in the proportion of 34% and 27% of total protein content, and denaturation temperatures are about 68 °C and 90 °C, respectively. The result indicated that adding the soy protein isolate could delay the thermal denaturation of meat protein, so the denaturation temperatures of C2 and C3 were higher than C1. Secondly, the G′ values of C1, C2 and C3 were slightly decreased from 52 °C to 58 °C, 53 °C to 59 °C, and 54 °C to 59 °C, respectively. The main reason was the protein network of previously formed was disrupted caused by the denaturation of the myosin tails. The results demonstrated that added soy protein isolate and treated by HPP processing could decline the pre-gel effects generated by the denaturation of myosin tails. Immediately, because of protein-protein interactions, the transformation from a viscous sol to an elastic gel network, as a result of a rapid increase in G′ occurred at approximately 58 °C as the temperature improved to 80 °C. On the other, the end G′ values was positively correlated with the hardness of cooked pork batters. The G′ values at 80 °C of C1, C2 and C3 were 33.31 kPa, 48.43 kPa and 44.08 kPa, respectively. We obtained that the result was in agreement with the result of hardness, C2 had the highest hardness.

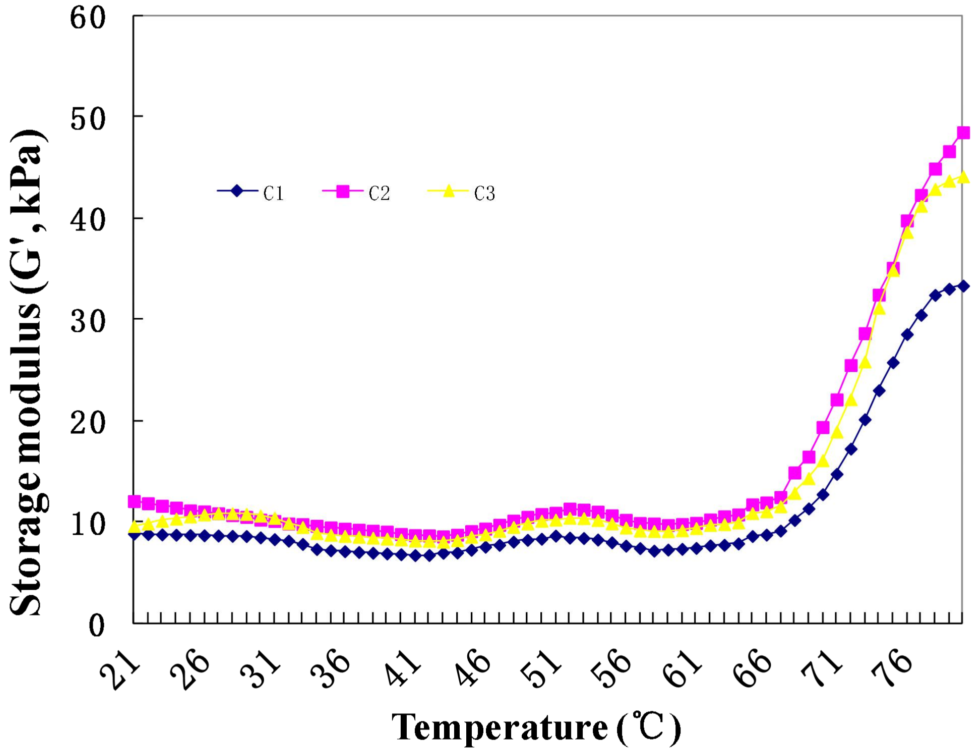

Fig. 3.4 Effect on rheological property of pork meat batters by high pressure processing with different soy protein isolate.

C1: 0% soy protein isolate; C2: 2% soy protein isolate; C3: 4% soy protein isolate.

3.8 Raman spectroscopy

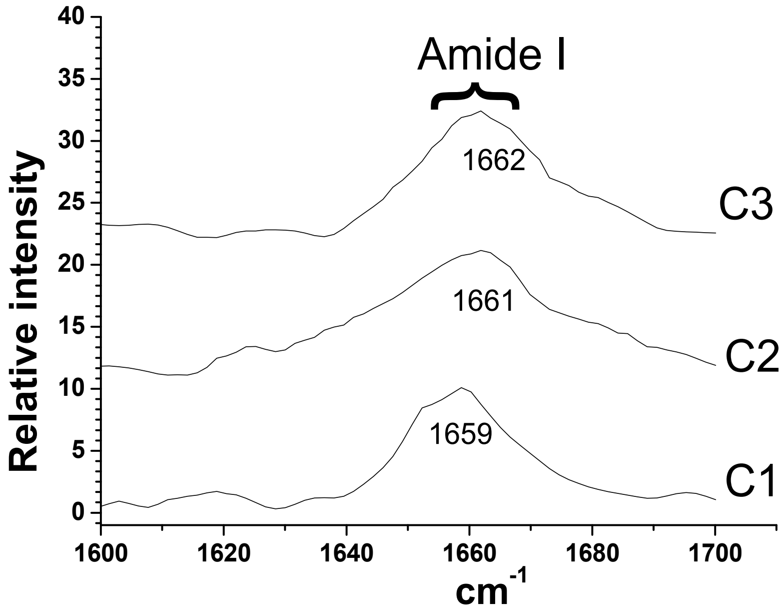

Fig. 3.5 - Raman spectra of pork meat batters by high pressure processing with different soy protein isolate in the region 1600–1700 cm^{-1}

C1: 0% soy protein isolate; C2: 2% soy protein isolate; C3: 4% soy protein isolate.

Table 3.5 - Effect on secondary structures (α-helix, β-sheet, β-turn, random coil, %) of pork meat batters protein by high pressure processing with different soy protein isolate

Sample	α-helix	β-sheet	β-turn	Random coil
C1	51.64 ± 1.85^{a}	20.83 ± 0.84^{b}	17.77 ± 0.38^{b}	11.42 ± 0.26^{c}
C2	45.62 ± 2.13^{b}	24.12 ± 0.76^{a}	18.58 ± 0.32^{a}	12.57 ± 0.21^{b}
C3	42.36 ± 1.92^{b}	25.26 ± 0.82^{a}	18.81 ± 0.41^{a}	14.22 ± 0.29^{a}

C1: 0% soy protein isolate; C2: 2% soy protein isolate; C3: 4% soy protein isolate.

Each value represents the mean ± SD, n = 4.

$^{a-c}$ Different parameter superscripts in the table indicate significant differences ($P < 0.05$).

The percentages of the secondary structures of proteins in the cooked batters treated by HPP with different soy protein isolate is shown in Table 3.5 and Fig. 3.5. We can observe that the strongest intensity bands of Amide I in the Raman spectra of C1, C2 and C3 were the 1659 cm^{-1}, 1661 cm^{-1}, and 1662 cm^{-1}, respectively. The content of the α-helix structure was significantly decreased ($P < 0.05$), and β-sheet, β-turn, random coil structures were significantly increased ($P < 0.05$) when added soy protein isolate. It is well known that the secondary structures of glycinin (11S) and β-conglycinin (7S) have three conformations, there are α-helix, β-sheet and β-turn. They contained about 35% of β-sheet structure, while the α-helix structure was negligible. The β-sheet and α-helix structures content of glycinin were about 56% and 16% through the circular dichroism spectrum measured, respectively. Thus, the main secondary structure of soy protein isolate is β-sheet. When added the soy protein isolate to pork meat batter, the α-helix structure was significantly decreased ($P < 0.05$). Due to the presence of soy protein in heated meat batters, the β-sheet and random coil structure contents were significantly ($P < 0.05$) increased and α-helix structure content was significantly ($P < 0.05$) decreased. The α-helix structure content was significantly ($P < 0.05$) decreased accompanied by a concomitant increase in β-sheet structure content in chopped pork batters with soy protein isolate. An increase in hydrogen bonding could induce more α-helix structure to change into β-sheet structure during protein denaturation with soy protein isolate. However, the random coil structure content was increased significantly ($P < 0.05$), the α-helix, β-turn, β-sheet structures were not significantly different ($P > 0.05$) with increasing the soy protein isolate content. In addition, the secondary structures are sensitive, when HPP processing induced changes in the hydrogen bonding scheme, and the increase of protein surface hydrophobicity and sulfhydryl content. So the α-helix structure content decreased significantly ($P < 0.05$) and β-sheet, β-turn and random coil structures content increased significantly ($P < 0.05$) in the pork meat batters protein by HPP processing with different soy protein isolate.

Conclusions in section 3

1. We obtained that the effect of high pressure and soy protein isolate combinations on the gel properties, rheological property and protein conformation of pork batters was significant differences.

2. The result showed that the pH, L^* and b^* values, cooking yield and hardness, cohesiveness and chewiness, G' values at 80 °C were significantly increased when added soy protein isolate, but the a^* values, TR, WR and FR were significantly decreased. The

TR, WR and FR, G' values at 80 °C were significantly decreased with the soy protein isolate increased.

3. The result of low field NMR exhibited that the batters with soy protein isolate had less water out the cooked pork batter and free water.

4. The α-helix structure changed into β-sheet, β-turn and random coil structures when added soy protein isolate, and the random coil structure content was increased significantly with the soy protein isolate increased.

5. We determined that the 2% soy protein isolate addition could improve the water holding capacity, texture and techno-functional properties of pork batters treated by high pressure.

SECTION 4.

EFFECTS OF SOY PROTEIN ISOLATE ON GEL PROPERTIES AND WATER HOLDING CAPACITY OF REDUCED-SALT PORK MYOFIBRILLAR PROTEIN UNDER HIGH PRESSURE PROCESSING

Myofibrillar protein is a salt-soluble protein, dissolvable in high-ionic-strength solutions (≥ 0.3 mol/L). It is the major functional protein in meat processing, and it determines the water-holding capacity and gel properties of emulsion meat products. NaCl plays a key role in the solubilization of myofibrillar proteins. Their solubility decreases as the level of NaCl decreases, which also lowers the water-holding capacity and texture properties of the gel. HPP could enhance the functional properties of myofibrillar proteins, and changes in protein conformational structures were caused. These findings could help to improve the gel properties of myofibrillar proteins. At low pressure levels (≤ 200 MPa), the solubility of myofibrillar proteins improved. Conversely, when the pressure was greater than 300 MPa, the solubility of myofibrillar proteins was reduced, and a large aggregate was formed. Therefore, HPP could affect molecular interactions and protein conformations, leading to denaturation, dissociation, and aggregation of myofibrillar proteins, and resulting in modified functional properties. The other, HPP could change the functional properties and structure of soy protein isolate. However, as far as we know, few studies reported the effect of soy protein isolate on techno-functional characteristics of reduced-salt pork myofibrillar protein under HPP. Therefore, for understanding the mechanism of improving the quality of reduced-salt pork batter through HPP and soy protein isolate, in this section, we evaluate the effects of soy protein isolate on gel properties and the water-holding capacity of reduced-salt pork myofibrillar protein under HPP, and thereby find a way to improve the techno-functional characteristics of reduced-salt (1% NaCl) pork myofibrillar protein.

4.1 Colour

The colour of cooked pork myofibrillar proteins with various amounts of soy protein isolate that was subjected to HPP processing is shown in Table 4.1. We can observe that the L^*, a^*, and b^* values significantly increased (P < 0.05) when soy protein isolate was added. However, the L^* values did not show significant differences (P < 0.05); meanwhile, the a^* and b^* values were significantly increased (P < 0.05) with the soy protein isolate increased.

Table 4.1 - Effect on colour (L^*, a^* and b^* values) of cooked pork myofibrillar proteins with various amounts of soy protein isolate under high pressure (200 MPa, 10±2 °C, 10 min).

Sample	L^* value	a^* value	b^* value
C1	73.44±0.67^b	-2.54±0.11^c	-5.23±0.13^c
C2	77.32±0.81^a	-0.95±0.10^b	-2.20±0.09^b
C3	79.01±0.72^a	0.63±0.09^a	0.32±0.11^a

C1, 0% soy protein isolate; C2, 2% soy protein isolate; C3, 4% soy protein isolate.
Each value represents the mean ± SE, n=4.
$^{a-c}$ Different superscripts in the same column indicate significant differences (P < 0.05).

We assumed the reason is that the structure of cooked pork myofibrillar proteins was improved when the soy protein isolate and 200 MPa treatment were combined, and a better gel structure has a higher L^* value. An increased/denser network structure causes a higher light reflectance, thereby an increasing lightness. The result implied that added 2% or 4% soy protein isolate and HPP combined could promote the myofibrillar proteins to form a better structure. In addition, because soy protein isolate was darker than the myofibrillar proteins, the a^* and b^* values decreased significantly (P < 0.05) when 2% and 4% soy protein isolate were added.

4.2 Cooking yield

Cooking yield is a key factor for determining the water-holding capacity of pork myofibrillar proteins during the heating processing. The cooking yield of pork myofibrillar proteins with various amounts of soy protein isolate under HPP is shown in Fig. 4.1. We can see that the cooking yield improved significantly (P < 0.05) when the soy protein isolate concentration was increased. It is well known that soy protein isolate could absorb water three times its own weight during the heating processing. The water- and fat-holding capacities of soy protein isolate increased following the HPP.

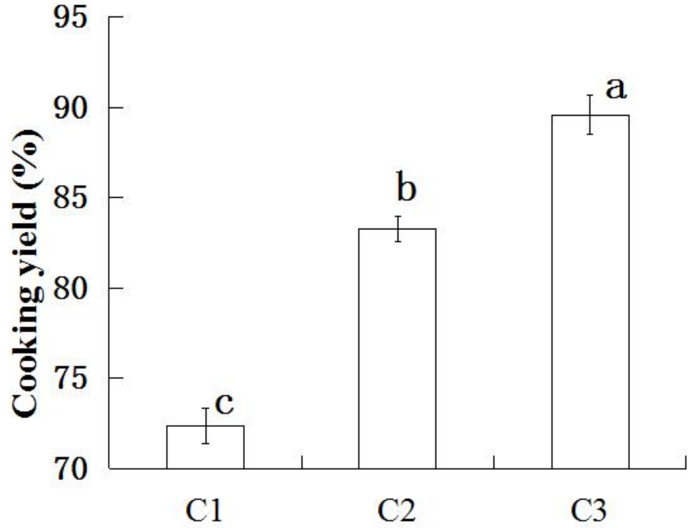

Fig. 4.1 - Effect on cooking yield of pork myofibrillar proteins with various amounts of soy protein isolate under high pressure processing (200 MPa, 10±2 °C, 10 min).
C1, 0% soy protein isolate; C2, 2% soy protein isolate; C3, 4% soy protein isolate.
Each value represents the mean ± SE, n=4.
[a-c] Different superscripts in the same column indicate significant differences (P < 0.05).

4.3 Texture measurement

Texture is an important indicator that can reflect the quality of meat products. The texture characteristics of cooked pork myofibrillar proteins with various amount of soy protein isolate by HPP processing is shown in Table 4.2. We found that the hardness, cohesiveness, and chewiness increased significantly (P < 0.05) when soy protein isolate was added. C1 had the lowest hardness or C3 had the highest hardness, whereas C2 had the highest springiness, cohesiveness, and chewiness, but the springiness of C1 and C3 were not significantly different (P > 0.05). Due to their participation in the network structure coupled to the myofibrillar proteins, added dried egg white proteins remarkably increased the hardness-cutting force and compression of the low-fat chicken meat gels treated by HPP. In addition, because the HPP-induced association between soybean and meat proteins caused a stiffening of the matrix, the hardness was improved significantly (P < 0.05) with the soy protein isolate concentration increasing from 2% to 4%. To the contrary, the springiness, cohesiveness, and chewiness were decreased significantly ($P < 0.05$) with the soy protein isolate concentration increasing from 2% to 4%. A possible reason is that excessive addition of soy protein isolate broke down the gel structure, which reduced the springiness, cohesiveness, and chewiness. Although the addition of soy protein isolate could improve the processing characteristics of pork myofibrillar proteins under HPP, the texture properties with 4% soy protein isolate were poorer than the sample of 2% soy protein isolate.

Table 4.2 - Effect on texture characteristics of cooked pork myofibrillar proteins with various amounts of soy protein isolate under high pressure (200 MPa, 10±2 °C, 10 min).

Sample	Hardness (g)	Springiness	Cohesiveness	Chewiness (g.mm)
C1	262.25 ± 6.22^{c}	0.826 ± 0.007^{b}	0.443 ± 0.03^{c}	95.96 ± 4.43^{c}
C2	335.82 ± 7.35^{b}	0.887 ± 0.012^{a}	0.527 ± 0.04^{a}	156.98 ± 4.36^{a}
C3	371.72 ± 5.58^{a}	0.832 ± 0.010^{b}	0.471 ± 0.02^{b}	145.67 ± 3.97^{b}

C1, 0% soy protein isolate; C2, 2% soy protein isolate; C3, 4% soy protein isolate.

Each value represents the mean ± SE, n=4.

$^{a\text{-}c}$ Different superscripts in the same column indicate significant differences ($P < 0.05$).

4.4 Total and reactive sulfhydryl groups

The change of total and reactive sulfhydryl groups is closely related to protein denaturation and reflects the alterations in protein structures (Omana, Plastow & Betti, 2011). Table 4.3 shows the effect of total and reactive sulfhydryl groups of myofibrillar proteins with various amounts of soy protein isolate under HPP. We can find that the total and reactive sulfhydryl groups improved significantly ($P < 0.05$) with the increase of soy protein isolate. The exposure of the buried sulfhydryl groups of myosin was enhanced at 150 MPa. The soy protein isolate contains a few of sulfhydryl groups, especially the glycinin, the six subunits of the glycinin molecule are all connected by an acidic subunit and an alkaline subunit with a disulfide bond, and each subunit also contains 2 or 3 cysteine and cystine side chain residues, and the reactive sulfhydryl content of soy protein isolate was significantly increased under 200–300 MPa and between 5 and 15 min. The free sulfhydryl content of glycinin gradually increased when treated under HPP processing from 0 to 600 MPa. Thus, we can assume that the total and reactive sulfhydryl groups were increased with increasing soy protein isolate concentration. In addition, more active sulfhydryl groups were exposed through protein unfolding after HPP, resulting in an increase of reactive sulfhydryl content when the protein concentration increased.

Table 4.3 - Effect on total and reactive sulfhydryl (μmol/g), surface hydrophobicity (μg) of pork myofibrillar proteins with various amounts of soy protein isolate under high pressure (200 MPa, 10±2 °C, 10 min).

Sample	Total sulfhydryl (μmol/g)	Reactive sulfhydryl (μmol/g)	Surface hydrophobicity (μg)
C1	57.17 ± 0.68^{c}	22.36 ± 0.35^{c}	14.54 ± 0.44^{b}
C2	66.23 ± 0.52^{b}	29.53 ± 0.42^{b}	18.32 ± 0.47^{a}
C3	73.51 ± 0.73^{a}	33.22 ± 0.38^{a}	19.25 ± 0.55^{a}

C1, 0% soy protein isolate; C2, 2% soy protein isolate; C3, 4% soy protein isolate.

Each value represents the mean ± SE, n=4.

$^{a\text{-}c}$ Different superscripts in the same column indicate significant differences (P < 0.05).

4.5 Surface hydrophobicity

Changes in surface hydrophobicity could reflect the myofibrillar protein conformation and structure. A higher S_0 value implied a stronger hydrophobic interaction of protein molecules. The effect of soy protein isolate on surface hydrophobicity of myofibrillar proteins treated under HPP processing is shown in Table 4.3. The surface hydrophobicity increased significantly ($P < 0.05$) when soy protein isolate was added, but the surface hydrophobicity showed no significantly differences (P > 0.05) with the increase of soy protein isolate from 2% to 4% under 200 MPa. We can prove that the surface hydrophobicity content of soy protein isolate increased significantly under 200-300 MPa. Thus, soy protein isolate and HPP processing might induce partial protein denaturation when combined, which improve the functional properties of proteins, such as gelling and water-holding capacity (Fig. 4.1 and Table 4.2).

4.6 Rheological measurement

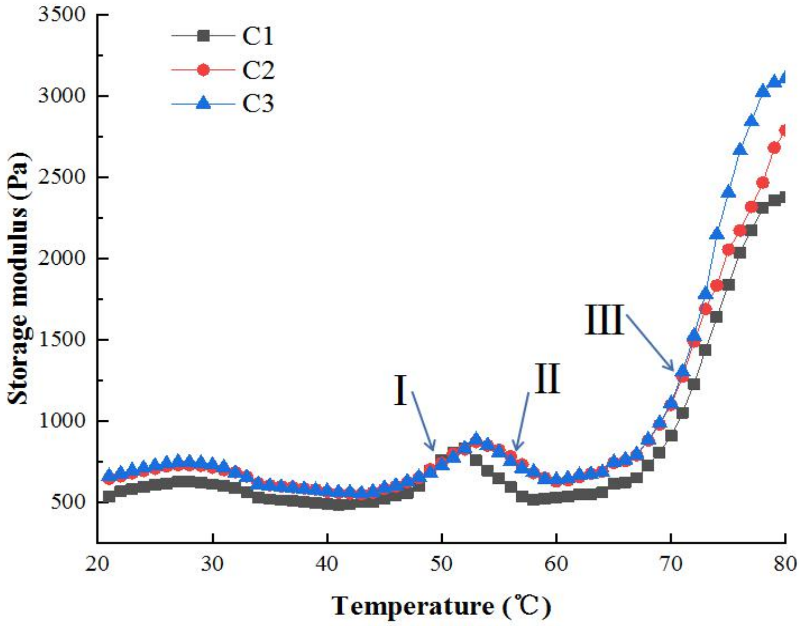

Fig. 4.2 The changes of storage modulus (G') of pork myofibrillar proteins with various amounts of soy protein isolate after high pressure during the heating processing (200 MPa, 10±2 °C, 10 min).

C1, 0% soy protein isolate; C2, 2% soy protein isolate; C3, 4% soy protein isolate.

I, the first phase; II, the second phase; III, the third phase.

It is well known that dynamic rheology is usually used to evaluate the viscoelastic properties of myofibrillar proteins. G' reflects the elastic changes of the gel, which should be analyzed considering springiness. The effect of soy protein isolate on the G' of pork myofibrillar proteins with various amounts of soy protein isolate after HPP processing during the heating is shown in Fig. 4.2. All the treatments exhibited a typical G' curve of pork myofibrillar proteins after HPP processing with three phases evident from 20 °C to 80 °C. In the first phase, the G' of C1, C2, and C3 were slightly elevated from 41 °C to 52 °C, 43 °C to 53 °C, and 43 °C to 53 °C, respectively. The results were caused by producing a larger number of unfolded myofibrillar and protein aggregation in the myofibrillar proteins solution after HPP processing was treated and the temperature improved. The denaturation temperatures of glycinin and β-conglycinin under 200 MPa were approximately 70 °C and 90 °C, respectively. Thus, adding soy protein isolate could enhance the heating stability of pork myofibrillar proteins and increase the denaturation temperature. In the second phase, the G′ values declined slightly from 53 °C to 58 °C (C1), and from 54 °C to 60 °C (C2 and C3), respectively. A possible reason is that the dissolution and/or denaturation of proteins first cause a denser matrix (a kind of a filling effect), and when the protein interaction takes place due to increase temperature, they are compressed and leave some voids, softening as result. Our findings indicated that soy protein isolate and HPP processing when combined could improve the stability of pre-gel of pork myofibrillar proteins. In the third phase, the G' of all treatments increased rapidly at about 60 °C as the temperature increased to 80 °C. A possible reason is that the protein–protein interactions were enhanced, which promoted a viscous sol transformed into an elastic gel network. At 80 °C, the G′ values of C1, C2, and C3 were 2380 Pa, 2791 Pa, and 3113 Pa, respectively. Some studies have found that the hardness of cooked gel was positively correlated with the end G′ values, resulting in C3 having the highest hardness.

4.7 Low-field NMR measurements

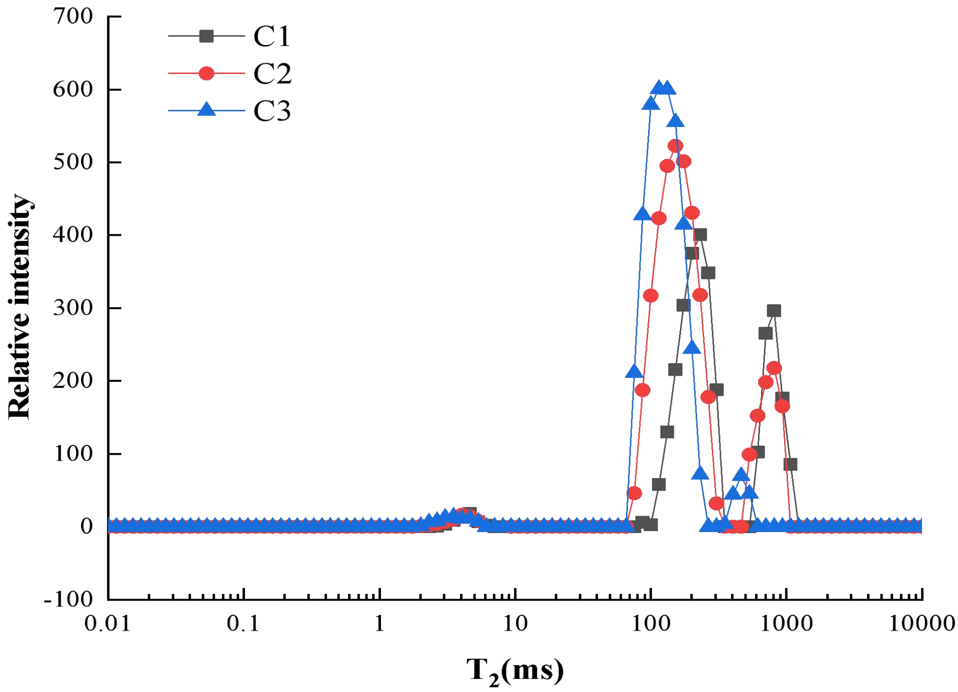

Fig. 4.3 The curves of relaxation time (T_2) of cooked pork myofibrillar proteins with various amounts of soy protein isolate under high pressure processing (200 MPa, 10±2 °C, 10 min).

C1, 0% soy protein isolate; C2, 2% soy protein isolate; C3, 4% soy protein isolate.

Low-field NMR has been part of the research on the distribution of water in meat products, and it has been a powerful tool for identifying water components. The water tightly associated with protein and macro-molecular constituents was labeled T_{2b}, and it represents about 1–4% of the total water in the meat system. The intra-myofibrillar water and water within the protein structure were named as T_{21}, which was a major component, and T_{22} possibly corresponds to extra-myofibrillar water. Fig. 4.3 and Table 4.4 showed the low-field NMR analysis result (relaxation time and peak ratio) of cooked pork myofibrillar proteins with various amounts of soy protein isolate under HPP processing (200 MPa, 10 min). We found that the initial relaxation times of T_{2b}, T_{21}, and T_{22} were shortened ($P < 0.05$) when soy protein isolate was added. The result means that the cooked pork myofibrillar proteins with various amounts of soy protein isolate treated by HPP could restrain the water tightly because of the changes in the fast-relaxing protein and slowly relaxing water protons. Because the soy protein isolate had excellent water- and fat-holding capacity, gelling, and structuring behavior, the initial relaxation times of T_{21} and T_{22} decreased significantly ($P < 0.05$) with soy protein isolate increase. The results were in agreement with the changes in cooking yield (Fig. 4.1). In addition, the 200 MPa treatment could significantly improve the emulsifying activity of soybean 11S globulins of soy protein isolate. The combination of soy protein isolate and HPP processing increased significantly ($P < 0.05$) the surface hydrophobicity and free SH content (Table 4.3). We established that these promoted a better gel structure of cooked pork myofibrillar protein formed during heating.

Table 4.4 - The initial relaxation time (T_2, ms) and peak ratio (P_2, %) of cooked pork myofibrillar proteins with various amounts of soy protein isolate under high pressure (200 MPa, 10±2 °C, 10 min).

Sample	Initial relaxation time (ms)			Peak ratio (%)		
	T_{2b}	T_{21}	T_{22}	P_{2b}	P_{21}	P_{22}
C1	2.65±0.07^{a}	97.72±1.76^{a}	832.21±6.63^{a}	1.75±0.05^{a}	72.35±1.18^{c}	25.65±0.35^{a}
C2	2.03±0.06^{b}	84.32±0.98^{b}	781.29±5.84^{b}	0.87±0.06^{b}	83.42±1.32^{b}	15.75±0.33^{b}
C3	1.95±0.07^{b}	75.63±1.14^{c}	715.68±7.18^{c}	0.72±0.06^{b}	90.90±1.04^{a}	8.45±0.37^{c}

C1, 0% soy protein isolate; C2, 2% soy protein isolate; C3, 4% soy protein isolate.

Each value represents the mean ± SE, n=4.

$^{a\text{-}c}$ Different superscripts in the same column indicate significant differences (*P* < 0.05).

The peak ratios of P_{2b} and P_{22} significantly decreased (P < 0.05) when soy protein isolate was added, whereas the peak ratios of P_{21} significantly increased (P < 0.05). That was in agreement with the results of initial relaxation times (Table 4.4) and cooking yield (Fig. 4.1). The result indicated that the water and protein tightly associated with each other, decreased the water loss during the heating. A possible reason is that the addition of soy protein isolate could increase the protein concentration, and the HPP processing increased the total and reactive sulfhydryl, surface hydrophobicity of soy protein isolate and myofibrillar proteins solution (Table 4.3), these induced a better gel structure was formed and reduced the fluidity of water. Therefore, added soy protein isolate could improve the water-holding capacity of pork myofibrillar proteins under HPP.

Conclusions in section 4

1. According to the result, we obtained that the treatment of pork myofibrillar proteins with soy protein isolate at different concentrations under high pressure (200 MPa, 10 min) could significantly affect its water-holding capacity, rheology, and gel properties.

2. The result showed that the surface hydrophobicity, total and reactive sulfhydryl of pork myofibrillar proteins increased when the addition of soy protein isolate, accompanied by a significantly increased in cooking yield, hardness, L^* value, and G' value at 80 °C.

3. When 4% soy protein isolate was added, the texture properties of cooked pork myofibrillar proteins were reduced.

4. The peak ratios of P_{2b} and P_{22} decreased significantly. Conversely, the peak ratio of P_{21} increased significantly with increasing soy protein isolate concentration.

5. The results suggested that the bound and free water declined, and the immobilized water increased. In conclusion, a combination of 2% soy protein isolate and 200 MPa pressure could improve gel properties of reduced-salt (1% NaCl) pork myofibrillar proteins.

SECTION 5.

TECHNO-FUNCTIONAL PROPERTIES, WATER DISTRIBUTION AND MOBILITY OF REDUCED-SALT PORK BATTERS WITH SOY PROTEIN ISOLATE AS AFFECTED BY PRESSURES

Reduced-salt meat products have been paid more and more attention by consumers in recent years, because about 20–30% of daily sodium consumption is from meat products. A larger number of methods have been used in emulsion meat products to reduce the sodium chloride content, such as HPP, and adding non-meat proteins. Through the results of above, we found that HPP and soy protein isolate combined could improve water holding capacity and texture properties of reduced-salt emulsion meat products and reduced-salt pork myofibrillar proteins. Pressure levels play an important role in the quality of emulsion meat products, such as, the changes in the quaternary structure of proteins are maintained by hydrogen bonds at low pressures (< 150 MPa), the tertiary structures might be affected by the formation of hydrophobic and ionic interactions at pressures (> 200 MPa), and the solubility of myofibrillar protein was increased under low pressures (≤ 200 MPa), opposite, the solubility was decreased accompanied by the large aggregate was formed under 300 MPa and over. As far as we know, a little study reported the effect of different pressures on techno-functional properties, water distribution and mobility of reduced-salt pork batters with soy protein isolate. Therefore, the aim of this section was to evaluate the colour, texture and rheological properties, and water distribution and mobility of reduced-salt pork batters (1% sodium chloride) with soy protein isolate (2%) treated by different pressures, and thereby to obtain a method to improve the water holding capacity and texture of reduced-salt pork batter.

5.1 pH

We received the following experimental data about the changes of pH in the cooked batters with soy protein isolate treated by different pressures from the Fig. 5.1

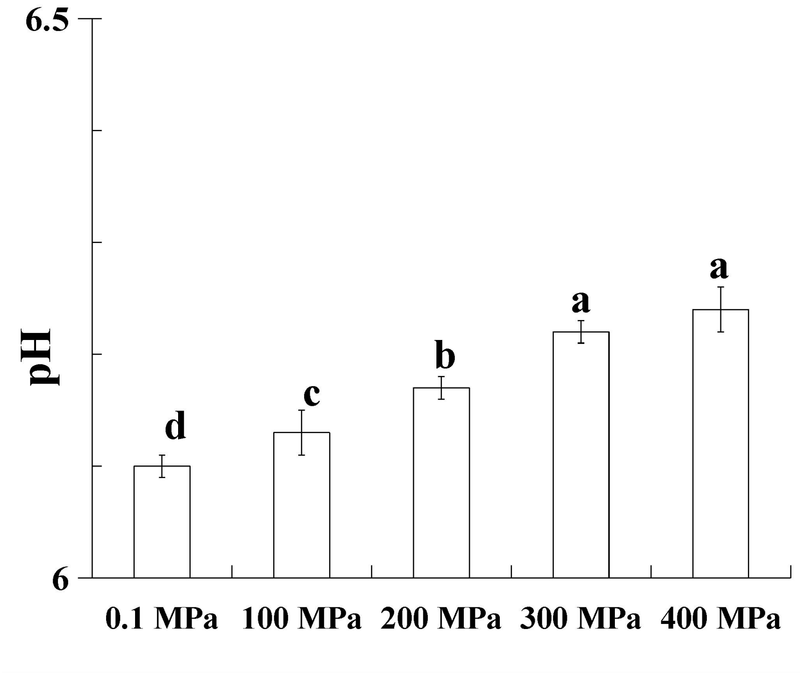

Fig. 5.1 pH of cooked batters with soy protein isolate treated by different pressures. Each value represents the mean ± SD, n = 4.

[a-d] Different parameter superscripts in the figure indicate significant differences ($P < 0.05$).

Compared with the 0.1 MPa, the pH of cooked batters with soy protein isolate treated by HPP was significantly increased ($P < 0.05$), except for the 100 MPa. The pH was significantly increased ($P < 0.05$) with increasing the pressures, but between the 300 MPa and 400 MPa were no significant differences ($P > 0.05$). We assume the reason for these findings might be that HPP treatment could induce protein unfolding, more alkaline groups were exposed, such as the thin proteins from the filament (actin, tropomyosin, troponin C and M-protein) were solubilized at 100 MPa, myosin light chains were released from myofibrils subjected to 100 MPa, whereas solubilization of myosin heavy chains required up to 300 MPa, these led to the pH was increased. The other, was the use of the HPP treatment and sea tangle powder combinations to reduce the phosphate in emulsion-type sausage, and found that the pH and protein solubility was increased after being subjected to HPP treatment. Due to the exposure of acidic groups were decreased, and the conformational changes of proteins associated with denaturation during the HPP, the pH of post-rigor meat has a slight increase after HPP treatment, and it was increased with the increase of the pressures.

5.2 colour

We received the following experimental data about the effect of colour in cooked batters with soy protein isolate treated by different pressures from Table 5.1.

Table 5.1 - The colour (L^*, a^* and b^* values) and cooking yield (%) of cooked batters with soy protein isolate treated by different pressures.

Sample	L^* value	a^* value	b^* value	Cooking yield (%)
0.1 MPa	58.65 ± 1.28^{b}	6.39 ± 0.32^{a}	13.46 ± 0.40^{a}	89.65 ± 0.48^{d}
100 MPa	59.46 ± 1.35^{b}	5.23 ± 0.48^{ab}	12.01 ± 0.52^{ab}	91.37 ± 0.35^{c}
200 MPa	62.35 ± 1.68^{a}	4.65 ± 0.49^{b}	11.26 ± 0.57^{b}	95.32 ± 0.31^{a}
300 MPa	63.66 ± 1.51^{a}	4.08 ± 0.29^{b}	11.37 ± 0.40^{b}	95.66 ± 0.33^{a}
400 MPa	61.19 ± 1.26^{ab}	3.70 ± 0.21^{bc}	10.87 ± 0.48^{b}	93.19 ± 0.46^{b}

Each value represents the mean ± SD, n = 4.

$^{a\text{-}d}$ Different parameter superscripts in the table indicate significant differences ($P < 0.05$).

Compared with the 0.1 MPa, the L^*, a^* and b^* values of 100 MPa were no significant differences ($P > 0.05$). Meanwhile, the L^* value was significantly increased ($P < 0.05$), a^* and b^* values were significantly decreased ($P < 0.05$) when the over 200 MPa, but all the L^*, a^* and b^* values of 200 MPa, 300 MPa and 400 MPa were no significant differences ($P > 0.05$). The main reason is possible that HPP processing can induce the change in molecular interactions (hydrogen bonds, hydrophobic interactions, sulfydryl and electrostatic bonds), protein conformation of myofibrillar protein and soy protein isolate, that presents altered functional properties, such as improved solubility, water holding capacity. In addition, HPP processing could change the oxidative states of the pigment myoglobin, and the occurrence of redox reaction can be visualized by L^*, a^* and b^* values.

5.3 Cooking yield

The cooking yield expresses the ability of comminuted meat products to hold water and fat, which is an important indicator of product quality. The cooking yield of the pork batters with soy protein isolate was significantly affected ($P < 0.05$) by different pressures (Table 5.1). Compared with the 0.1 MPa, the cooking yield of the batters treated by different pressures was significantly increased ($P < 0.05$). We presumed the reason might be that HPP can unfold the myofibrillar proteins and soy protein isolate, and increased the pH (Fig. 5.1), these prompt more proteins to dissolve and form a three dimensional network that entraps water molecules during heating. The cooking loss of reduced-fat and reduced-salt pork sausage emulsions was significantly decreased after treatment by 200 MPa (2 min) was reported. We assumed the reason is that an increase in water and fat holding capacity in pork meat batter treated by HPP, because the increase of the solubilization and denaturation of muscle proteins. The other, soy protein isolated has a good moisture and oil adsorption and keeping ability. Therein, the emulsifying activity of 11S globulin was significantly increased at 200 MPa, which enhanced the fat holding capacity of soy protein isolate during processing. In addition, the cooking yield was significantly increased ($P < 0.05$) from 0.1 MPa to 200 MPa, and was no significant differences ($P > 0.05$) between 200 MPa and 300 MPa, while the cooking yield was significantly decreased ($P < 0.05$) at 400 MPa. The main reason is that the myofibrillar proteins were partially unfolded and formed a gel with many filaments and irregular cavities at 100 MPa. At 200 MPa, the smallest particle size of myofibrillar proteins were generated and formed a gel with denser and homogeneous network. Moreover, above 300 MPa, the myofibrillar proteins were denatured excessively, and formed a gel with larger cavities and heterogeneous. Thus, excessive pressure lowered the water and fat holding capacity of the pork batters.

5.4 TPA

The texture of emulsion meat products is an important factor to determine whether the consumers purchased it or not. As shown in Table 5.2, we observed the TPA of the cooked batters with soy protein isolate was significantly affected by the different pressures. Compared with the 0.1 MPa, the hardness, springiness, cohesiveness and chewiness of cooked batters treated by different pressures were significantly increased ($P < 0.05$). This might be that HPP processing induced texture modifications have been used to improve myofibrillar proteins and their gel-forming properties, such as stronger protein-protein interactions, which could prompt the muscle protein gel to form a better texture. The reason is that the myofibrillar proteins, such as myosin and actin, were more salt soluble when reduced-salt beef sausage batters were subjected to HPP at 200 MPa than the untreated batter. Which caused that the hardness, springiness, cohesiveness, chewiness, and resilience of chicken meat batters produced by HPP were significantly increased than the 0.1 MPa. The gels from tilapia muscle proteins had a harder texture treated by 200 MPa, mainly because of partly depolymerized, unfolded, aggregated and denatured the extracted proteins under 200 MPa, which caused the changes in water distributions, formed new protein components, and solubilization or denaturation of myofibrillar proteins. In addition, the hardness, springiness, cohesiveness and chewiness were significantly increased ($P < 0.05$) from 0.1 MPa to 200 MPa, and was not significant differences ($P > 0.05$) between 200 MPa and 300 MPa, while the hardness, springiness, cohesiveness and chewiness were significantly decreased ($P < 0.05$) at 400 MPa. Thus, the 200 MPa and 300 MPa had the highest hardness, springiness, cohesiveness and chewiness among the treatments. Due to mild pressure ($\leq$ 300 MPa) treatment before heating could significantly enhance the heat gelation of myosin, since myofibrillar protein denatured and stretched, which made its structure destabilized, exposed more hydrophobic groups and sulfhydryl groups, these exposed hydrophobic residues were cross-linked to aggregate, sulfhydryl groups were reacted to form disulfide bonds during heating. It is well known that over 200 MPa treatment, the protein extractability was decreased significantly in the meat batters, and caused protein denaturation and/or aggregation, which limited their functionalities. The other, moderate HPP treatment at 200 MPa led to a significant increase in free SH content, while at a pressure above 200 MPa, SH contents of soy protein isolate were progressively and significantly decreased with increasing pressures. Thus, the texture properties of 300 MPa was not significant differences ($P > 0.05$)

compared with the 200 MPa. Meanwhile, stronger pressure (> 300 MPa) resulted in the depolymerization of myofibrillar protein, and its irregular aggregation formed a uniform gel network structure and induced the reduction of hardness and chewiness.

Table 5.2 TPA of cooked batters with soy protein isolate treated by different pressures.

Sample	Hardness (N)	Springiness	Cohesiveness	Chewiness (N.mm)
0.1 MPa	47.61±0.84^{c}	0.814±0.007^{c}	0.624±0.010^{c}	26.18±0.92^{d}
100 MPa	49.46±0.75^{b}	0.842±0.008^{b}	0.650±0.09^{b}	31.67±1.05^{b}
200 MPa	53.21±0.98^{a}	0.863±0.009^{a}	0.687±0.007^{a}	35.68±0.89^{a}
300 MPa	52.24±0.91^{a}	0.860±0.010^{a}	0.676±0.009^{a}	34.87±0.86^{a}
400 MPa	50.27±0.76^{b}	0.822±0.007^{c}	0.639±0.008^{bc}	30.39±0.98^{c}

Each value represents the mean ± SD, n = 4.

$^{a\text{-}d}$ Different parameter superscripts in the table indicate significant differences ($P < 0.05$).

5.5 Rheology property

Storage modulus (G′) is a key index for rheology properties of meat proteins, which is correct with the elastic behavior of the solid-like components and directly affects the gel properties of meat batter. We obtained the following experimental data about the effects of G′ on raw batters with soy protein isolate treated by different pressures from 20 °C to 80°C in Fig. 5.2.

All the curves of the changes on G′ were similar, and they had a typical curve of dynamic rheology with three phases caused by meat proteins denaturation during heating. Firstly, the initial G′ value was increased from 8.22 kPa to 18.50 kPa with the increase of pressure, the result indicated that HPP processing could induce the denaturation of meat proteins and soy protein isolate, and form a weakened gel structure, which led to the G′ value to increase.

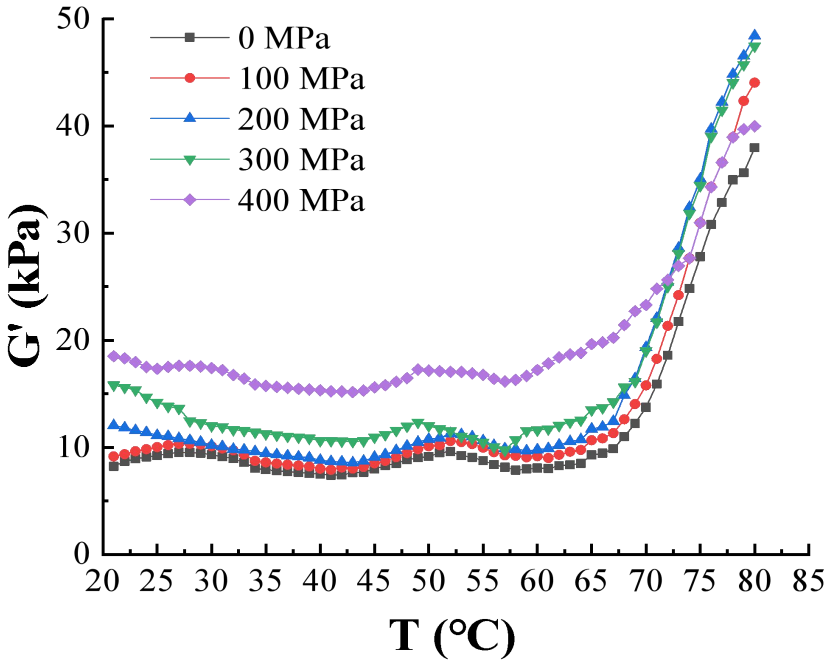

Fig. 5.2 The changes in storage modulus (G', kPa) of cooked batters with soy protein isolate treated by different pressures

After that, due to the pork back-fat melting and the protein unfolding with increasing the temperature, the G′ value was slightly decreased from 20 °C to 41 °C (0.1 and 100 MPa), and 43 °C (200, 300 and 400 MPa), respectively. In the second phase, because of the protein-protein interactions and weak gel formation, the G′ values of 0.1 and 100 MPa were slowly increased from 42 °C to 52 °C, the 200 MPa was slowly increased from 44 °C to 52 °C, while the 300 MPa and 400 MPa were slowly increased from 44 °C to 49 °C, respectively. Immediately, the G′ values of 0.1 MPa. 100 MPa and 200 MPa were slowly decreased from 53 °C to 58 °C, while 300 MPa and 400 MPa were slowly increased from 50 °C to 57 °C, respectively. The different changes were caused by the different pressures, because the myosin has partial denaturation when the pressures were 300 MPa and over. The other, some researchers reported that soy protein isolate and HPP processing combined can decrease the pre-gel effects produced by the denaturation of myosin tails. Thus, HPP processing and added soy protein isolate could lower the change in G′ values, which are caused by the denaturation of the myosin tails. In the third phase, a rapid increase in G′ value was occurred from approximately 58 °C to 80 °C, that implying the viscous sol was transformed into an elastic gel. In addition, the differences were possible caused by the denaturation of β-conglycinin (7S), it is present in the proportion of 27% of total soy protein isolate content, and the denaturation temperature is about 68 °C. Herein, the techno-functional properties of β-conglycinin was affect by the different pressures, such as the water and fat holding capacity of soy protein isolate were increased after the HPP treatment. The G′ values at 80 °C of 0.1, 100, 200, 300 and 400 MPa were 37.97, 44.05, 48.44, 47.47 and 39.99 kPa, respectively, the result was in agreement with the result of hardness (Table 5.2). It is well known that the G′ values were higher, the gel was firmer.

5.6 NMR spin-spin relaxation time (T2)

The effects of initial relaxation time and peak ratio of cooked batters with soy protein isolate treated by different pressures are shown in Fig. 5.3 and Table 5.3.

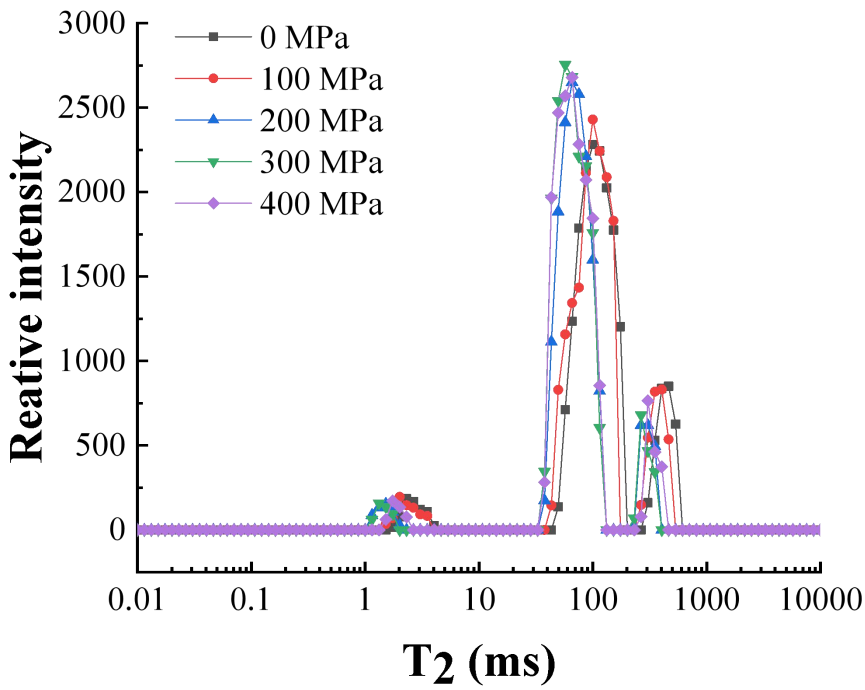

Fig. 5.3 Curves of relaxation time (T_2) in cooked batters with soy protein isolate treated by different pressures

The proton transverse relaxation time and peak ratio (T_2) can provide the information to evaluate the distribution and mobility of water in the cooked batter. Three peaks of T_{2b}, T_{21} and T_{22} are observed from 0.01 ms to 10000 ms in the inversion map of the nuclear magnetic intensity, and they are named bound water, immobilized water and free water respectively (Fig. 5.3). The relaxation population of T_{2b} is centered at approximately 0-10 ms, it is assigned to water tightly associated to protein and macro-molecular constituents through the hydrogen bonding to polar groups. The relaxation population of T_{21} is located at 35-100 ms, it is represented to intra-myofibrillar water and water within the protein structure, indicating this water is loosely bound in the sol matrix. Opposite, T_{22} corresponds to extra-myofibrillar water and centered at approximately 100-350 ms.

Table 5.3 - The initial relaxation time (ms) and peak ratio (%) of cooked batters with soy protein isolate treated by different pressures

Sample	Initial relaxation time (ms)			peak ratio (%)		
	T_{2b}	T_{21}	T_{22}	P_{2b}	P_{21}	P_{22}
0.1 MPa	1.82±0.12^{a}	47.74±1.36^{a}	308.67±3.55^{a}	1.20±0.13^{a}	84.32±2.62^{bc}	14.42±0.61^{a}
100 MPa	1.51±0.15^{b}	42.23±1.52^{bc}	285.53±4.19^{b}	1.27±0.15^{a}	85.07±2.08^{bc}	13.86±0.83^{a}
200 MPa	1.12±0.15^{c}	37.25±1.59^{d}	232.87±4.68^{d}	0.96±0.12^{a}	91.87±2.45^{a}	8.31±0.80^{c}
300 MPa	1.18±0.13^{c}	36.44±1.61^{d}	235.36±4.88^{d}	1.07±0.18^{a}	92.09±2.51^{a}	7.80±0.75^{c}
400 MPa	1.46±0.12^{b}	40.31±1.39^{c}	263.27±4.31^{c}	1.23±0.16^{a}	87.36±2.17^{b}	11.35±0.68^{b}

Each value represents the mean ± SD, n = 4.

$^{a-d}$ Different parameter superscripts in the table indicate significant differences ($P < 0.05$).

In this section, compared with the 0.1 MPa, the initial relaxation times of T_{2b}, T_{21} and T_{22} were quicker ($P < 0.05$) in the cooked batter treated by different pressures, implying that the water in the cooked batters treated by different pressures was tied closer. In addition, the samples of 200 and 300 MPa had the shortest initial relaxation times of T_{2b}, T_{21} and T_{22} ($P < 0.05$) among the treatments. The result indicated that the cooked batters treated by 200 MPa and 300 MPa had good gel structure, this was in agreement with the result of cooking yield and texture properties (Table 5.1 and 5.2). All the peak ratios of T_{2b}

in the cooked batters were not significant differences ($P > 0.05$). The samples of 200 and 300 MPa had the largest peak ratio of P_{21} and the smallest peak ratio of P_{22}, meanwhile, the samples of 0.1 MPa and 100 MPa had the smallest peak ratio of P_{21} and the largest peak ratio of P_{22}. We suggested the main reason is possible that the solubility of myofibrillar protein was increased, more hydrophobic residues and sulfydryl groups of raw batter were exposed when treated by HPP processing, which was favour of forming good structure and enhancing water holding capacity. The other reason is that due to the protein-protein interaction at pressure 400 MPa and above were formed at the expense of protein-water interactions and caused the aggregation, which could decrease the solubility of myofibrillar protein. Thus, excessive pressures led to the gel structure being worse and more water moving, causing the free water to increase and the immobilized water to decrease.

Conclusions in section 5

1. The result showed that the effect of different pressures on the pH, colour, water holding capacity, texture properties, and rheological property of pork batters with soy protein isolate were significantly different.

2. The pH, cooking yield, hardness, springiness, cohesiveness and chewiness, and G′ values at 80 °C were significantly increased in the batters treated by 100 MPa and over.

3. The L^*, a^* and b^* values were no significant differences between 0.1 MPa and 100 MPa. The L^* value, cooking yield, texture properties, G′ values at 80 °C and the peak ratio of P_{21} were the highest when treated by 200 and 300 MPa.

4. We suggested that the techno-functional properties of Reduced-salt pork batters could be improved when treated by 200 and 300 MPa.

SECTION 6.

EFFECTS OF SOY PROTEIN ISOLATE AND HIGH PRESSURE COMBINED ON GEL QUALITIES OF PASTEURIZED REDUCED-SALT PORK BATTER STORED AT COLD TEMPERATURE

For improving the quality and shelf-life of reduced-salt emulsified meat products, some methods have been used, such as using the HPP processing, adding non-meat protein, etc. HPP has been wildly used to change the techno-functional characteristics and inhibitory microorganisms of emulsified meat products, but the processing does not affect the flavors. HPP and heated combinations could improve the bactericidal effects and techno-functional properties of meat products. The other, due to soy protein isolated has good water and fat adsorption and keeping ability during the machining process, and cheaper, it as the most commonly used non-meat protein is widely added in the emulsified meat products. However, the effect of gel properties, microbe and TBARS from pasteurized reduced-salt pork batter treated by HPP with different soy protein isolate levels during cold storage were not studied. Therefore, according to the "Production process and operation key points of produce reduced-salt pork batter using the technology of high pressure and soy protein isolate combination" (Appendix A), we produced the reduced-salt pork batter using the technology of HPP and soy protein isolate combination and stored at 2±2 °C for 60 days, to determine the effect of soy protein isolate and HPP combined on product quality of pasteurized reduced-salt pork batter.

6.1 Storage loss

The effect of storage loss on the pork batters treated by HPP with different soy protein isolate during cold storage is presented in Table 6.1. We found that all the storage loss of pork batters were increased significantly ($P < 0.05$) with increasing the storage time. We assumed this is possible that due to the presence of oxygen, the protein and fat of pork batters were oxidized during cold storage, resulting in decreasing protein processing performance and destruction of the network structure in meat products, leading to the water- and fat- holding capacity were decreased. At the same storage time, the storage loss of pork batters without soy protein isolate was increased significantly ($P < 0.05$) compared with that of 2% soy protein isolate. Some studies have reported that soy protein isolate has good water- and fat- holding capacity, gel property, an so on. The functional characteristics of meat products were improved when the soy protein isolate was added. In addition, the emulsifying activity of soy 11S globulin was increased significantly treated under 200 MPa, thus, the result enhanced the water- and fat- holding capacity of soy protein isolate. The other, the storage loss of pork batter with 2% soy protein isolate stored at the 60th day was lower than the pork batter without soy protein isolate at the 30th day. Hence, adding 2% soy protein isolate could improve the stability of water and fat from pork batters during cold storage.

Table 6.1 - Effect on storage loss (%) and total plate count (CFU/g) of pasteurized reduced-salt pork batters treated by high pressure processing with different soy protein isolate during cold storage

Sample	Storage loss (%)	Total plate count (CFU/g)
C1st	0^{d}	NB
C30th	$4.27±0.44^{b}$	NB
C60th	$7.19±0.53^{a}$	$6.21×10^{3}$
T1st	0^{d}	NB
T30th	$1.68±0.17^{c}$	NB
T60th	$2.05±0.22^{c}$	$1.63×10^{2}$

C1st, 0% soy protein isolate and stored at the 1st day; C30th, 0% soy protein isolate and stored at the 30th day; C60th, 0% soy protein isolate and stored at the 60th day; T1st, 2% soy protein isolate and stored at the 1st day; T30th, 2% soy protein isolate and stored at the 30th day; T60th, 2% soy protein isolate and stored at the 60th day.

Each value represents the mean ± SE, n = 4.

$^{a\text{-}d}$ Different parameter superscripts in the figure indicate significant differences ($P < 0.05$).

6.2 Total plate count

The result of the total plate count of pork batters treated by HPP with different soy protein isolate during cold storage is presented in Table 6.1. All the total plate counts of pork batters were not significantly different ($P > 0.05$) at the 1st and 30th days, and increased significantly ($P < 0.05$) at the 60th day. It is well known that HPP could retard the growth and reproduction of microorganisms, and also kill them under suitable conditions, but limit to kill spores. In this section, the pork batters were treated at 200 MPa for 10 min, and following heated at 80 °C, however, the processing conditions can not kill all spores. Thus, the total plate count of pork batter was increased significantly during cold storage. The other, at the 60th day, the total plate count of pork batters without soy protein isolate was increased significantly ($P < 0.05$) compared with that of 2% soy protein isolate. The reason is possible that the pork batters without soy protein isolate had a higher storage loss than that of 2% soy protein isolate (Table 6.1), the higher apparent moisture of pork batters increased the $A_{w,}$ and it promoted the growth and reproduction of microorganisms.

6.3 TBARS

As shown in Fig. 6.1, the TBARS of pork batters treated by HPP with different soy protein isolate during cold storage was affected. We can observe that all the TBARS of pork batters were increased significantly ($P < 0.05$) with the increase in cold storage time. It is well known that HPP could form the free radical and promote fat oxidation. Especially, some studies have reported that the influence of HPP on fat stability of meat products is related to oxygen, the composition of meat products and temperature. Such as, the pressure over 300 MPa at room temperature aggravated the rate of lipid oxidation, and the TBARS value of peroxides from meat tissue was higher than that of unpressurized lipids. Meanwhile, at the same storage time, the TBARS of pork batters without soy protein isolate was increased significantly ($P < 0.05$) compared with that of 2% soy protein isolate. The result was related to the result of storage loss (Table 6.1), more free water was in favour of the formation of free radicals during cold storage, and increased the TBARS value.

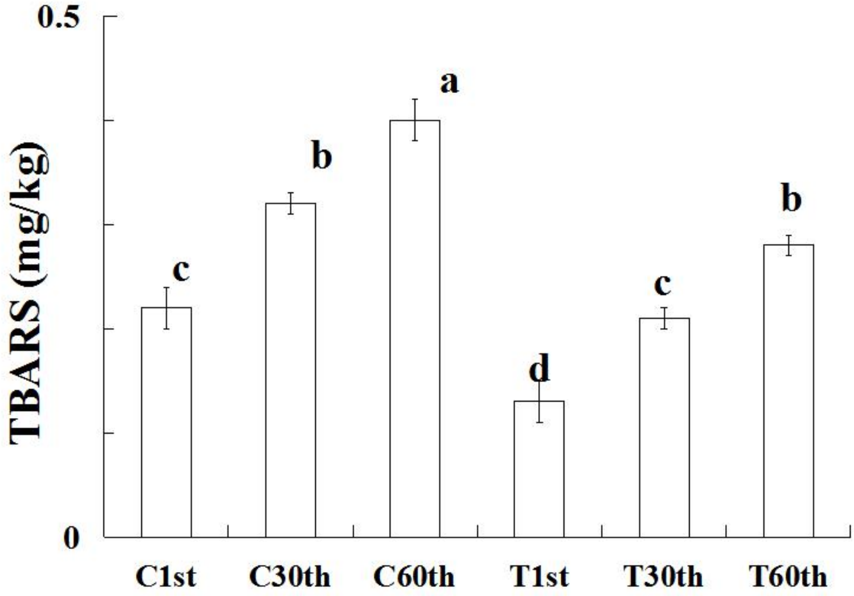

Figure 6.1 - Effect on TBARS (mg/kg) of pasteurized reduced-salt pork batters treated by high pressure processing with different soy protein isolate during cold storage.

C1st, 0% soy protein isolate and stored at the 1st day; C30th, 0% soy protein isolate and stored at the 30th day; C60th, 0% soy protein isolate and stored at the 60th day; T1st, 2% soy protein isolate and stored at the 1st day; T30th, 2% soy protein isolate and stored at the 30th day; T60th, 2% soy protein isolate and stored at the 60th day.

Each value represents the mean ± SE, n = 4.

[a-d] Different parameter superscripts in the figure indicate significant differences (P < 0.05).

6.4 pH

The changes in pH of pork batters treated by HPP processing with different soy protein isolate during cold storage is shown in Fig. 6.2. With the increase of cold storage time, the pH of pork batters without soy protein isolate was increased significantly (P < 0.05), meanwhile, the pork batters with 2% soy protein isolate was not significantly different (P > 0.05) at the 1st and 30th days, and increased significantly (P < 0.05) at the 60th day. The result was agreement with the result of TBARS (Fig. 6.1). It is well known that the lipid oxidation and protein oxidation could produce an alkaline substance, which improved the pH of pork batters during cold storage. The other, due to the microorganisms breaking down the proteins and fats to obtain energy and nutrition, their growth and reproduction improved the pH of pork batters during cold storage. At the same storage time, the pH of pork batters without soy protein isolate was decreased significantly (P < 0.05) compared with that of 2% soy protein isolate. Our previous papers found that because soy protein isolate has a higher pH value, more alkaline residues of pork batters were exposed which were treated by HPP and soy protein isolate combinations, so adding soy protein isolate could increase the pH of pork batters and reduced-salt pork myofibrillar protein. On the whole, the ascending range of pH from pork batters without soy protein isolate was larger than that of 2% soy protein isolate.

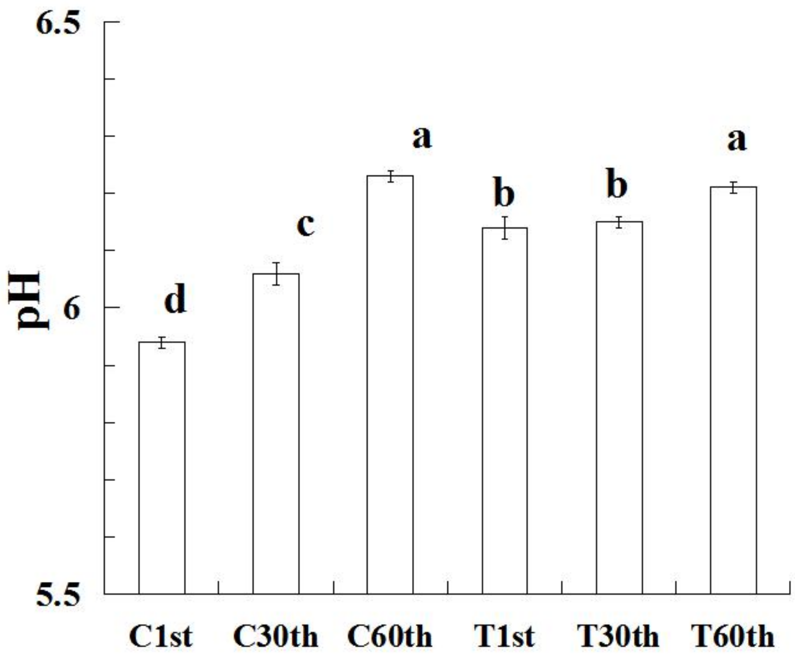

Fig. 6.2 Effect on pH of pasteurized reduced-salt pork batters treated by high pressure processing with different soy protein isolate during cold storage.

C1st, 0% soy protein isolate and stored at the 1st day; C30th, 0% soy protein isolate and stored at the 30th day; C60th, 0% soy protein isolate and stored at the 60th day; T1st, 2% soy protein isolate and stored at the 1st day; T30th, 2% soy protein isolate and stored at the 30th day; T60th, 2% soy protein isolate and stored at the 60th day.

Each value represents the mean ± SE, n = 4.

$^{a\text{-}d}$ Different parameter superscripts in the figure indicate significant differences (P < 0.05).

6.5 Instrumental colour

The changes in colour (L^*, a^* and b^* values) of pork batters treated by HPP with different soy protein isolate during cold storage is shown in Table 6.2. With the increase of storage time, all the L^* values of pork batters were increased significantly ($P < 0.05$), except for the T1st and T30th. That was in agreement with the result of storage loss (Fig. 6.1). The L^* and b^* values of frankfurters were increased, the a^* value was lowered when soy protein isolate was added. The changes in L^* values were caused by the light reflection. More moisture on the surface of pork batters caused the increase of L^* values. Thus, the L^* values were increased with increasing the cold storage time. The a^* value of pork batters without soy protein isolate was decreased significantly ($P < 0.05$) with the increase in cold storage time, while the pork batters with 2% soy protein isolate was not significantly different ($P > 0.05$). Meanwhile, the b^* values were not changed ($P > 0.05$) during cold storage. The loss of a^* value is caused by the oxidation of myoglobin during the cold storage. In addition, due to the pork back-fat globules were covered by soy protein isolate and formed smaller back-fat globules after adding the soy protein isolate, so the pork batters with 2% soy protein isolate had a better water holding capacity and texture properties, the gel structure was stability than the pork batters without soy protein isolate. We can establish that the colour of the pork batters with 2% soy protein isolate was more stable than the pork batters without soy protein isolate.

Table 6.2 - Effect on colour (L^*, a^* and b^* values) of pasteurized reduced-salt pork batters treated by high pressure processing with different soy protein isolate.

Sample	L^* value	a^* value	b^* value
C1st	58.92 ± 1.30^{c}	6.27 ± 0.60^{a}	9.52 ± 0.30^{b}
C30th	62.28 ± 1.56^{b}	5.12 ± 0.47^{b}	9.14 ± 0.36^{b}
C60th	67.87 ± 1.31^{a}	3.22 ± 0.53^{c}	8.72 ± 0.47^{b}
T1st	62.35 ± 1.68^{b}	4.65 ± 0.49^{bc}	11.26 ± 0.57^{a}
T30th	63.05 ± 1.53^{b}	4.01 ± 0.59^{bc}	10.85 ± 0.50^{a}
T60th	67.74 ± 1.81^{a}	3.85 ± 0.49^{bc}	10.32 ± 0.53^{a}

C1st, 0% soy protein isolate and stored at the 1st day; C30th, 0% soy protein isolate and stored at the 30th day; C60th, 0% soy protein isolate and stored at the 60th day; T1st, 2% soy protein isolate and stored at the 1st day; T30th , 2% soy protein isolate and stored at the 30th day; T60th, 2% soy protein isolate and stored at the 60th day.

Each value represents the mean ± SE, n = 4.

$^{a\text{-}c}$ Different parameter superscripts in the figure indicate significant differences ($P < 0.05$).

6.6 Texture

The changes in the texture of pork batters treated by HPP with different soy protein isolate during cold storage is shown in Table 6.3.

Table 6.3. Effect on texture of pasteurized reduced-salt pork batters treated by high pressure processing with different soy protein isolate.

Sample	Hardness (N)	Springiness	Cohesiveness	Chewiness (N mm)
C1st	47.32±1.12^{d}	0.837±0.008^{b}	0.641±0.005^{c}	27.05±0.85^{c}
C30th	50.27±1.08^{c}	0.814±0.006^{c}	0.620±0.004^{d}	25.37±0.64^{d}
C60th	56.30±1.33^{a}	0.681±0.007^{d}	0.582±0.006^{e}	22.31±0.76^{e}
T1st	53.21±0.98^{b}	0.863±0.009^{a}	0.687±0.007^{a}	35.68±0.89^{a}
T30th	53.48±1.09^{b}	0.857±0.005^{a}	0.684±0.005^{a}	34.50±0.77^{a}
T60th	55.21±1.20^{a}	0.834±0.007^{b}	0.665±0.006^{b}	31.42±0.92^{b}

C1st, 0% soy protein isolate and stored at the 1st day; C30th, 0% soy protein isolate and stored at the 30th day; C60th, 0% soy protein isolate and stored at the 60th day; T1st, 2% soy protein isolate and stored at the 1st day; T30th , 2% soy protein isolate and stored at the 30th day; T60th, 2% soy protein isolate and stored at the 60th day.

Each value represents the mean ± SE, n = 4.

$^{a\text{-}d}$ Different parameter superscripts in the figure indicate significant differences ($P < 0.05$).

With the increase in cold storage time, all the hardness values of pork batters were increased significantly ($P < 0.05$), except the T1st and T30th. This is because that the storage loss was increased significantly with the increase of cold storage time (Table 6.1), the moisture content of pork batter was decreased, which led to the hardness value increased. Due to the changes in moisture content of T1st and T30th being limited, their hardness values were not significantly different (P > 0.05). Meanwhile, the springiness, cohesiveness, and chewiness were decreased significantly (P < 0.05) with the increase in cold storage time, except for the T1st and T30th. The result was caused by the increase of fat oxidation, microbial, pH and water loss. The other reason is that HPP processing (200 MPa) and soy protein isolate combined could improve the hardness, springiness, cohesiveness and chewiness of pork batters, which was beneficial to keep the stability of texture properties. Thus, the result indicated that the texture of the pork batters with 2% soy protein isolate was more stable than the pork batters without soy protein isolate.

6.7 Technology of reduced-salt pork batters using soy protein isolate and high pressure processing

The results of the study were used in the development of technology on soy protein isolate and HPP combined to produce the pasteurized reduced-salt pork batter, and its shelf-life was not less the 60 days during cold storage. The technological scheme for the production of the developed product is shown in Fig. 6.3.

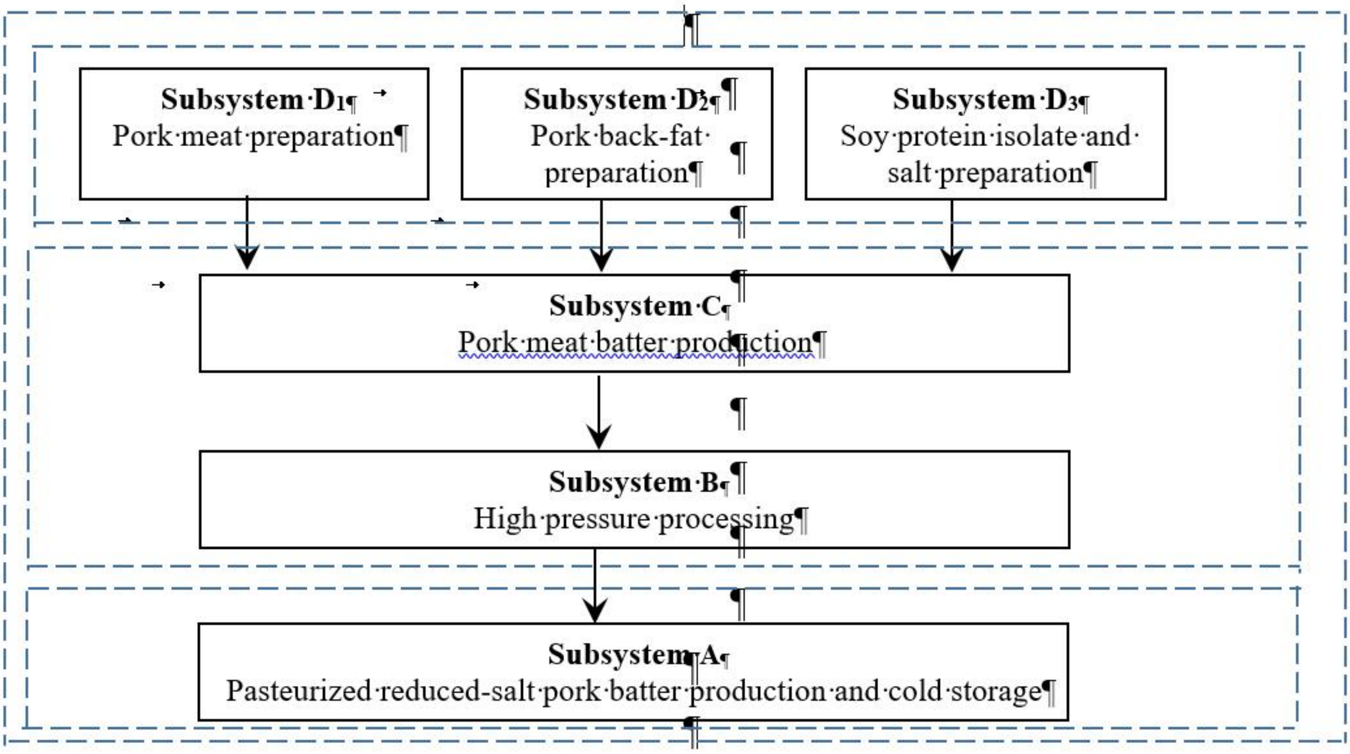

Fig. 6.3 Technological scheme of production in pasteurized reduced-salt pork batter using the technology of soy protein isolate and HPP combined

As it can be seen from Fig. 6.3, the general system of technological process consists of such stages with allocation of subsystems of such hierarchy: (D_1, D_2, D_3) → (C)→ (B) → A.

The first stage is preparatory. It includes subsystems D_1 "Pork meat preparation", D_2 "Pork back-fat preparation", and D_3 "Soy protein isolate and salt preparation". The second stage is processing technology. It is the main one. It includes subsystem C "Pork meat batter production" and subsystem B "High pressure processing". The third stage is production. It includes subsystems A "Pasteurized reduced-salt pork batter production and cold storage".

The selection of these subsystems has a clear structural and logical justification and is associated with the presence of individual operations and the ability to control quality at each stage of production.

The implementation of the system as a whole ensures the functioning of its individual structural elements in accordance with the goal, Table 6.4.

Table 6.4 – The composition of the technological system and the purpose of its structural operation elements

Subsystem	Name of subsystem	Aim of subsystem functioning
A	Pasteurized reduced-salt pork batter production and cold storage	Obtaining pork batter with reduced salt, specified structural, mechanical properties and quality indicators.
B	High pressure processing	Increasing the solubility of muscle and soy proteins, improving their technological and functional properties, to reduce the salt content.
C	Pork meat batter	Chopping and mixing the pork

	production	meat, back-fat, water, soy protein isolate and salt, forming a uniform emulsion meat batter.
D_1	Pork meat preparation	Provide muscle proteins for forming the structure of pork meat batter
D_2	Pork back-fat preparation	Provide animal fat for improving the gel properties and water holding capacity of pork meat batter
D_3	Soy protein isolate and salt preparation	Provide non-meat protein for improving the gel properties, and lowering salt content of pork meat batter; extract the salt-soluble proteins to enhance their processing properties

All the stages can be implemented both in the small and the big food enterprises. Therein, the preparatory stage (subsystems D_1, D_2 and D_3) is the foundation of product success; the subsystem B and C determine the salt content and physicochemical parameters of the finished product and its range. Therefore, it is closely related to subsystem A.

Ensuring a high level of subsystem A functioning guarantees the quality of finished pasteurized reduced-salt pork batter using the technology of soy protein isolate and HPP combined, as well as their stable quality indicators in the process of storage and sale.

Technological scheme of pasteurized reduced-salt pork batter using the technology of soy protein isolate and HPP combined is presented at Fig. 6.4.

6.8 Socio-economic effectiveness of scientific and technical developments and implement the results of work in practical production

Apply the technology of soy protein isolate and HPP combined to produce the pasteurized reduced-salt pork batter, its cooking yield, texture properties and gel structure were significantly increased, and successfully reduced the salt content to 1%. The technology has carried out in mass production in 3 factories in China (Henan Zhongpin Food Industry Co. LTD; Hua County Ji Xianda Food Co. LTD; Nanjing Huang Professor Science and Technology Food Co. LTD) to produce reduced-salt (1% sodium chloride) emulsion meat products, such as meatballs, Taiwan sausage and breakfast sausage. According to our counted and evaluation, the socio-economic effectiveness of scientific and technical developments and implement the results of work in practical production was as follows:

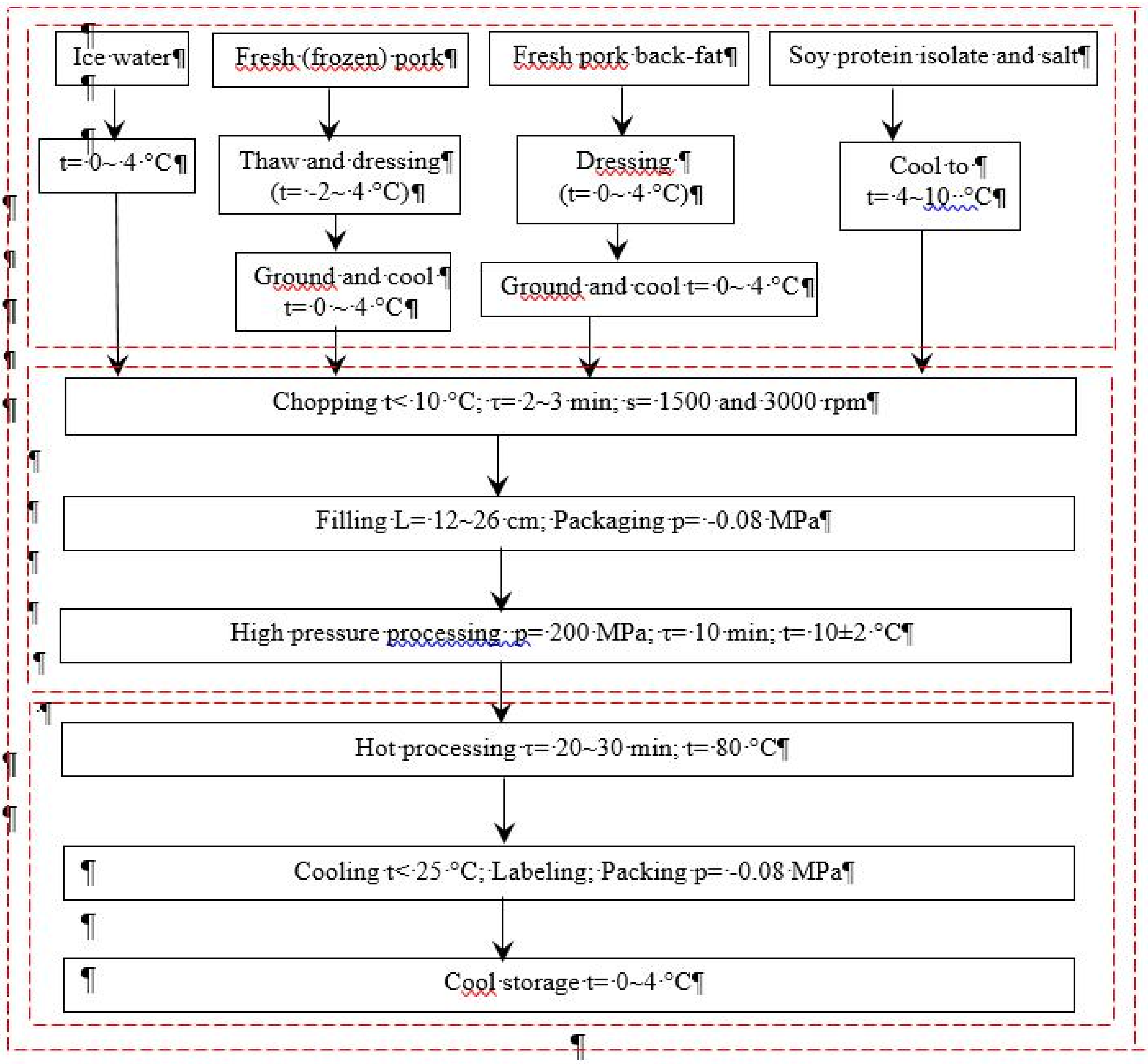

Fig. 6.4. Technological scheme of pasteurized reduced-salt pork batter using the technology of soy protein isolate and HPP combined.

First, the HPP as a green technology, producing 1 ton of product can reduce electricity by about 20~30 kilowatt hours and 0.8~1.5 tons running water compared with the traditional processing, it is beneficial to reduce the consumption of fossil energy, water and carbon dioxide emissions, thus, reduce the greenhouse effect and waste water, and contribute to environmental protection. Meanwhile, the electricity bill was reduced by \$2.00~\$2.50 per ton.

Second, the salt content of this product is approximately 1%, which is about 40% lower than that of traditional products. According to our calculations, when consumers eat 100g of this type product, which can reduce their salt intake by 0.8g, and the content of protein is increased by 0.60%~0.85%. Thus, an improvement in the health of consumers as a result of the use of this type products with low salt content and high protein content is observed.

Third, it takes 5%~8% less time to produce a ton of product, resulting in higher productivity, increased workshop and equipment utilization, and reduced workshop and equipment depreciation by \$1.00~\$2.20 per ton. The other, on this basis, labor costs are reduced by \$6.00~\$8.00 per ton.

Fourth, based on data from 3 factories, the cooking yield of meatballs was increased by 4.65%~ 5.53%, the raw material cost of meatballs is \$1880 per ton in China, which reduces production costs by \$87.42~\$103.96 per ton; the cooking yield of Taiwan sausage was increased 3.66%, the raw material cost of Taiwan sausage is \$1950 per ton in China, which reduces production costs \$71.37 per ton; the cooking yield of breakfast sausage was increased 4.60%, the raw material cost of breakfast sausage is \$1900 per ton in China, which reduces production costs \$87.40 per ton.

Finally, based on the above, producing 1 ton of meatballs can save \$96.42~\$118.66; producing 1 ton of Taiwan sausage can save \$80.37~\$96.07; producing 1 ton of Taiwan sausage can save \$96.42~\$103.12. The technology has achieved very good economic benefits for the companies.

Conclusions in section 6

1. We found that the effect of soy protein isolate levels and storage times on the storage loss, total plate count, TBARS, pH, colour, hardness, springiness, cohesiveness, and chewiness of pasteurized reduced-salt pork batter treated by high pressure processing were significantly different.

2. The water- and fat- holding capacity, a^* value, springiness, cohesiveness, and chewiness of pork batters were decreased significantly with the increase of storage time, while the microbial, fat oxidation, pH and hardness were increased significantly.

3. Compared with the sample with 2% soy protein isolate, the gel structural and colour stability, and oxidative stability were lowered during the cold storage.

4. We obtained that the cold storage performances of pasteurized reduced-salt pork batters treated under 200 MPa could be improved when adding 2% soy protein isolate.

5. Apply the technology of soy protein isolate and HPP combined to produce the pasteurized reduced-salt pork batter, its cooking yield, texture properties and gel structure were significantly increased, and successfully reduced the salt content to 1%. The technology has carried out mass production in 3 factories in China to produce reduced-salt (1% sodium chloride) emulsion meat products, such as meatballs, Taiwan sausage and breakfast sausage. Producing 1 ton of meatballs can save \$96.42~\$118.66; producing 1 ton of Taiwan sausage can save \$80.37~\$96.07; producing 1 ton of Taiwan sausage can save \$96.42~\$103.12.

CONCLUSIONS

1. Systematically analyzed the influence of HPP processing on meat and meat products, soybean protein isolate, and reduced-salt meat products, and the influence of soybean protein isolate on meat and meat products based on previous studies, found that the use of HPP processing, and the addition of soybean protein isolate could lower the salt of emulsion meat products.

2. Systematically studied the effect of soy protein isolate on the water holding capacity and texture properties of pork batters (1% sodium chloride) treated under 200 MPa, analysis of the impact of the changes in soy protein isolate on improving water retention and texture. Obtained that added 2% and 4% soy protein isolate could significantly increase cooking yield and hardness, cohesiveness and chewiness, and the cooked pork batter had less water out and free water, and the 2% soy protein isolate addition could improve the water holding capacity and texture of pork batters treated under 200 MPa.

3. Systematically investigated the changes of gel characteristics, rheological property and protein secondary structure attributes of pork meat batters (1% sodium chloride) treated under 200 MPa with different soy protein isolate (0%, 2%, 4%). Obtained that the pH, L^* and b^* values, emulsified stability, and G' values at 80 °C were significantly increased when added soy protein isolate. The TR, WR and FR, G' values at 80 °C were significantly decreased with the soy protein isolate increased. Added soy protein isolate and treated by HPP delayed the thermal denaturation of meat protein and declined the pre-gel effects generated by the denaturation of myosin tails from 53 °C to 59 °C. The α-helix structure changed into β-sheet, β-turn and random coil structures when added soy protein isolate. Especially, the content of random coil structure was significantly increased from 11.42±0.26% to 14.22%±0.29 with soy protein isolate increased. Thus, the gel and rheological properties of pork meat batters could be improved by changing the protein conformation when added to 2% soy protein isolate.

4. Analysis of the effects of different pressures (0.1-400 MPa) on the pH, gel properties, rheology, water distribution and mobility of reduced-salt pork batters (1%

sodium chloride) with 2% soy protein isolate. Found that the pH, cooking yield, hardness, springiness, cohesiveness and chewiness, and G′ values at 80 °C were significant increased in the batters treated by 100 MPa and over. The L^*, a^* and b^* values were not significantly differences between 0.1 MPa and 100 MPa. The L^* value, cooking yield, texture properties, G′ values at 80 °C and the peak ratio of P_{21} were the highest when treated by 200 and 300 MPa, implying that lowered the water mobility and increased the immobilized water. These results showed that the techno-functional properties of reduced-salt pork batters could be improved when treated by 200 and 300 MPa.

5. Systematically investigated the effects of HPP and soy protein isolate combinations on gel properties and water holding capacity of reduced-salt pork myofibrillar protein. The surface hydrophobicity, total and reactive sulfhydryl of pork myofibrillar proteins increased when the addition of soy protein isolate, accompanied by a significantly increased cooking yield, hardness, L^* value, and G' value at 80 °C. When 4% soy protein isolate was added, the texture properties of cooked pork myofibrillar proteins were reduced. The rheological findings indicated that the thermal stability of the myofibrillar protein increased when soy protein isolate was added. The initial relaxation time of T_{2b}, T_{21}, and T_{22} decreased when soy protein isolate increased; meanwhile, the peak ratio of P_{21} increased significantly, implying that water had lower mobility. Thus, the combination of 2% soy protein isolate and 200 MPa pressure could improve gel properties of reduced-salt (1% NaCl) pork myofibrillar proteins by increasing the surface hydrophobicity, total and reactive sulfhydryl groups.

6. The technology of 2% soy protein isolate and 200 MPa pressure combinations was applied to the production of pasteurized reduced-salt pork batter. The result has shown that the water- and fat- holding capacity, a^* value, springiness, cohesiveness, and chewiness of pork batters were decreased significantly with the increase of storage time, while the microbial, fat oxidation, pH and hardness were increased significantly. the total plate count of samples were not detected before the 30th day, while increased significantly at the 60th day. The range of storage loss, microbial, TBARS, pH, colour and texture properties variations from the samples without soy protein isolate were larger than that of 2%. Thus, 2% soy protein isolate and 200 MPa pressure combinations could improve the

gel properties, water- and fat- holding capacity, and reduce the microbial reproduction of pasteurized reduced-salt pork batter during cold storage.

7. Based on the results of theoretical, experimental studies, and pilot tests in the factory, the “Production process and operation key points of produce reduced-salt pork batter using the technology of HPP and soy protein isolate combination” was formulated.

8. The technology of HPP and soy protein isolate combination have carried out mass production in 3 factories in China to produce reduced-salt (1% sodium chloride) emulsion meat products, such as meatballs, Taiwan sausage and breakfast sausage. Producing 1 ton of meatballs can save \$96.42~\$118.66; producing 1 ton of Taiwan sausage can save \$80.37~\$96.07; producing 1 ton of Taiwan sausage can save \$96.42~\$103.12.

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APPENDICES

APPENDIX A

OPERATION KEY POINTS

NO：2020071601

Production process and operation key points of produce reduced-salt pork batter using the technology of HPP and soy protein isolate combination

Version number A

Date posted 20200716

Maker Yanping Li

Unit Henan Zhongpin Food Industry Co. LTD

China Henan

Henan Zhongpin Food Industry Co. LTD

APPENDIX B

HZFI Controlled document	NO：2020071601 Commencement date：20200716	
Production process and operation key points of produce reduced-salt pork batter using the technology of HPP and soy protein isolate combination	Page 1	Total of 4 pages

1. Objective：

Standardize product production and ensure product quality.

2. Application scope:

It is suitable for the reduced-salt pork batter using the technology of HPP and soy protein isolate combination in meat products factory of Henan Zhongpin Food Industry Co. LTD.

3. Product formula:

All the raw pork batters were prepared with 100 kg pork meat, 20 kg pork back-fat, 18.38 kg ice water, 1% NaCl, 2% soy protein isolate. Therein, the raw pork is *longissimus dorsi* (moisture < 72%; protein > 21%; pH, 5.65-5.75); the protein content of soy protein isolate > 90%).

4. Process content:

4.1 Technological process:

Raw materials receive → Thaw → Dressing → Ground meat→ Chopping* → Filling → Packaging → High pressure processing* → Hot processing → Cooling → Labeling →

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Packing → Warehousing.

Note: * indicates the key process

4.2.1 Raw material reception and dressing:

Requirements to choose fresh (frozen) pork as raw materials. Frozen cut meat should be frozen well, thawed at 18±2 °C, and the center temperature should be controlled at 0~4 °C after thawing. Fresh meat should be pre-cooled until the central temperature drops to 6 °C before it can be put into use. After thawing, it should be repaired in time to remove lymph, lesions, blood stasis, floating hair, tendons, broken bones, surface air-dry layer, impurities, etc., and the temperature of meat should be controlled within 0~6 °C.

4.2.2 Ground meat

After removing of the fat and other non-muscle material, the *longissimus dorsi* of pork was ground using a meat grinder with a 6 mm holes plate (MGB-120, China). The meat grinder is required to operate normally, and the meat blade is sharp to ensure that the granularity of the meat is obvious.

4.3.2 Chopping

The ground meat, NaCl and 1/2 ice water was chopped (1500 rpm) for 30 s; and then

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added pork back-fat chopped (1500 rpm) for 30 s; prior to finishing with a high speed (3000 rpm) emulsification for 60 s, and the 1/2 ice water was continue to add for keep the final temperature less than 10 °C.

4.3.4 Filling and vacuum packing

Immediately after chopping, the batter was stuffed by a vacuum stuffer, in 24~40 mm diameter edible collagen sausage casings. Pork batters were hand linked at 12~26 cm intervals, and weighed. Finally, the batters were vacuum packed for subsequent pressure processing.

4.3.5 High pressure treatment

The batters or myofibrillar proteins were treated by different pressures using a high pressure vessel, the temperature was controlled through a thermo-stating circulator water bath. The high pressure procedure was as follows: pressure, 200 MPa; time, 10 min; temperature, 10±2 °C. The compression rate was approximately 3 MPa/s, and the decompression step was reached immediately (< 3 s).

4.3.6 Heating and cooling

APPENDIX E

HZFI Controlled document	NO：2020071601	
	Commencement date：20200716	
Production process and operation key points of produce reduced-salt pork batter using the technology of HPP and soy protein isolate combination	Page 4	Total of 4 pages

All batters were heated at 80 °C for 30 min in the water bather until the internal temperature 72 °C. Immediately, the cooked batters were cooled to 20 °C by running water and stored at 4 °C.

4.3.7 Labeling, packing and warehousing.

Cool products dry or dry the surface moisture of the bag, label to be correct, firm, the net content of the whole box shall not have negative deviation.The packaged products should be stored in the warehouse at 0~4 °C, and the shelf life is 60 days.

APPENDIX F

CERTIFICATE OF IMPLEMENTATION OF RESEARCH RESULTS

Application Testify

Application enterprise	Nanjing Huang Professor Science and Technology Food Co. LTD
Postal address	6 Tongwei Road, Xuanwu District, Nanjing City, Jiangsu Province, China
Starting and ending time	MAY, 5, 2020 - now
Contact Person	Xu Qingfen (+086 13903927596)

Our enterprise have utilized the technology of high pressure processing and soy protein isolate combined to reduce the sodium chloride addition in roast sausage and Baoxin pork meatball, the technology can reduce the contents of salt in roast sausage from 1.52% to 1.03%, and the cooking yield increased by 4.73%; it can reduce the contents of salt in Baoxin pork meatball from 1.63% to 1.15%, and the cooking yield increased by 5.06%. In our factory, the type of emulsion meat products were produced 13 tons per day.

Nanjing Huang Professor Science and Technology Food Co. LTD

NOV, 22, 2021

APPENDIX G

Application Testify

Application enterprise	Hua County Ji Xianda Food Co. LTD
Postal address	200 meters south of the intersection of Renmin Road and Xiangjiang Road, Hua County New District, Anyang City, Henan Province, China
Starting and ending time	OCT, 10, 2020 - now
Contact Person	Xu Qingfen (+086 13903927596)

Our company have applied the "Technology of reduced-salt meat batters using soy protein isolate and high pressure processing combination". The technology could effectively reduce the contents of salt and fat in meatballs and breakfast sausage. Therein, the contents of salt and fat in meatballs were 0.96% and 5.20%, respectively, the content of protein was increased by 0.85%, the cooking yield was increased by 4.65%, and now produce 6 tons per day; the contents of salt and fat in breakfast sausage were 0.98% and 4.60%, respectively, the content of protein was increased by 0.60%, the cooking yield was increased by 3.82%, and the now produce 4.5 tons per day. The technology has achieved very good economic benefits for our company.

Hua County Ji Xian Da Food Co. LTD.

DEC, 6, 2021

APPENDIXH

Application Testify

Application enterprise	Henan Zhongpin Food Industry Co. LTD
Postal address	21 Changshe Road, Changge, Henan Province, China
Starting and ending time	Jun, 10, 2020 - now
Contact Person	Wang Rui (+086 15038977822)

The technology of produce reduced-salt emulsion meat products through high pressure processing and soy protein isolate combination have been applied in our factory. It can reduce the content of salt and cooking loss in Chinese meatball and Taiwan sausage. Such as, the content of salt in Chinese meatball was 0.97%, the cooking loss was decreased by 5.53%; the content of salt in Taiwan sausage was 0.78%, the cooking loss was decreased by 3.663%. Now, Chinese meatball and Taiwan sausage are produce 12 tons per day.

Henan Zhongpin Food Industry Co. LTD

DEC, 2, 2021